SAFETY EVALUATION OF NEW SUNSCREEN ACTIVE

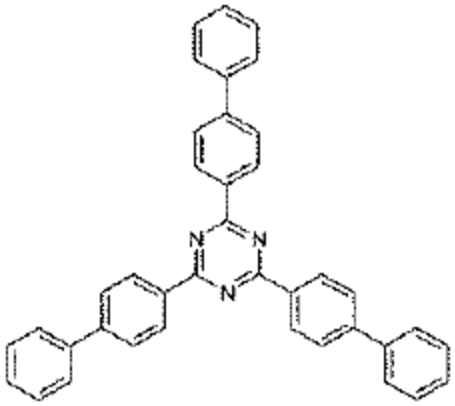
Tris-biphenyl triazine

Sponsor: BASF Australia Pty Ltd
Consultant: s47G [redacted]
Submission no. OM-2015-00223-1
TRIM no.: R15/838791
Report Date: 3 November 2015
Evaluator: s22 [redacted]

IDENTITY AND PHYSICOCHEMICAL PROPERTIES

Name: tris-biphenyl triazine (AAN)
Trade names: Tinosorb A2B
INCI name: tris-biphenyl triazine
Other names: **ETH50**, C-801, FAT 65'080, FAT 65'080/A, FAT 65'080/B, FAT 65'082/B, 2,4,6-Tris(p-biphenylyl)-s-triazine, Tinosorb A 2B
Chemical name: 1,3,5-Triazine, 2,4,6-tris([1,1'-biphenyl]-4-yl)-
Empirical formula: $C_{39}H_{27}N_3$
CAS No.: 31274-51-8
Molecular weight: 537.66
Appearance: white solid
Melting point: 281.3°C
pH: 10.5-12.0
Density: 1.256 g/cm^3 at 20°C
Water solubility: Insoluble (<0.03 µg/L at 21°C)
Log Pow: > 5.6 (calculated from the individual solubility in n-octanol and in water)
Particle size distribution: Commercial product - d(0.5) 109 nm, d(0.9) 175 nm.
Specification d(0.5)= 100-110 nm.
Proposed Use: UVA filter at a maximum concentration of 10%

Structure:



UV Spectrum: 290 to 340 nm

CONCLUSIONS AND RECOMMENDATION

Tris-biphenyl triazine is a UV filter that has been on the accepted list of UV filters in the EU since August 2014 at a concentration of up to 10% in products (not in sprays).
The sponsor has submitted a package of data containing studies on tris-biphenyl triazine that conform to GLP and OECD standards or acceptable guidelines regarding conduct of studies (includes QA statement).

Studies submitted in support of this application cover all elements of the stated guidelines, except for specific interaction data, a long-term dermal carcinogenicity bioassay (justification accepted), and a repeat dose toxicity study in a non-rodent species.

The reproductive toxicity study was only a preliminary study with a low number of animals and only one generation. However no indication was found that there is any effect on reproductive hormones, organs or activity.

No definable toxicity in animal studies (repeat dose and reproductive toxicity, and genotoxicity) at 500 mg/kg/day. The threshold dose for definable toxicity was above 500 mg/kg/day. Studies with animals and humans showed that tris-biphenyl triazine was not a skin irritant or sensitising agent, and it was not a phototoxic or photosensitising agent.

Toxicokinetic data indicated that systemic exposure following oral or dermal administration of tris-biphenyl triazine was very low. This impacts on the interpretation of oral toxicity studies and estimated safety margin.

Systemic exposure (based on dose/body weight) to tris-biphenyl triazine in animal studies at 500 mg/kg/day PO was likely to be above expected exposure in humans following normal use of a sunscreen with 10% tris-biphenyl triazine.

Justification for an absence of a dermal carcinogenicity bioassay was based on minimal human exposure to percutaneous tris-biphenyl triazine, an absence of alert features for the molecule, the molecule's stability, an absence of genotoxicity, an absence of sensitisation activity, an absence of photo-toxicity and photosensitisation, an absence of non-neoplastic changes in repeat-dose toxicity studies (although there was extremely low systemic exposure following oral dosing and the studies were of relatively short duration), and an absence of adverse effects on reproductive function and hormonal balance. The likelihood of tris-biphenyl triazine being carcinogenic would be low to negligible based on the information provided.

Inflammation of the lung after inhalation for 4 hours of nano-sized partcles of tris-biphenyl triazine occurred in rats. Although it is possible that this reaction does not occur in humans using spray formulations (since the use of the spray is expected to produce particles of a size that may not reach the lung), due to lack of repeat-dose inhalation toxicity testing, the use in sprays is not approved until further information is available. Due to the risk of toxicity after inhalation, in order for tris-biphenyl triazine (10%) to be approved for use in sprays, the Sponsor must:

- provide a 28-day repeated dose inhalation study, including tissue distribution and systemic toxicity, determination of an LOAEL and NOAEL and possible recovery (this kind of study was also requested by the Scientific Committee on Consumer Safety (SCCS; see page 42 of Appendix 1), and

- commit to ensure that the mean particle diameter of tris-biphenyl triazine when delivered via spray is >20 μm.

Overall, in the data provided tris-biphenyl triazine displayed a lack of definable toxicity in the studies presented at systemic exposure levels likely to be above those anticipated to occur in humans following dermal exposure. On this basis **tris-biphenyl triazine is acceptable for use as an active ingredient in sunscreen products at a concentration of up to 10%, not including products used in sprays.**

ASSESSMENT

The Sponsor provided studies conducted using test material with large particle sized (<14.5 µm) as well as nano-sized particle sizes (<175 nm).

Study	Material particle size	
	<175 nm material particle size	**<175 nm material particle size**
In vitro percutaneous absorption assay with rat skin	4.78%	12.77%
In vitro percutaneous absorption assay with human skin	0.20%	0.57%
In vitro percutaneous absorption assay with pre-damaged human skin	0.76%	-
Absorption data for single dermal dose in rats (*in vivo*)	-	0.11%
Absorption data for single oral dose in rats (*in vivo*)	0.06%	0.73%
Acute oral toxicity in rats	LD_{50} >1000 mg/kg	LD_{50} >2000 mg/kg
Acute dermal toxicity in rats	-	LD_{50} >2000 mg/kg
Acute inhalation toxicity in rats	LC_{50} >0.4976 mg/L air [1] or >4000 mg/kg	-
Primary skin irritation in rabbits	-	Non-irritant
Primary eye irritation in rabbits	-	Slight irritation
Skin sensitisation (local lymph node assay) in mice	-	Non-sensitiser (10%)
Photo-toxicity and photo-allergenicity in guinea pigs	-	Negative (10%)
Photo-toxicity and photo-allergenicity in humans	Negative (9.9%)	-
Genotoxicity Ames assay *in vitro*	-	Negative
Cultured human lymphocytes chromosomal aberration assay *in vitro*	-	Negative
Chinese hamster V79 cells chromosomal aberration assay *in vitro* (+ irradiation)	-	Negative
In vitro mammalian mutation (TK locus)	-	Negative
Photomutagenicity assay (Ames) *in* vitro (± S9)	-	Negative
In vivo mouse micronucleus test (IP route)	Negative	Negative
In vivo unscheduled DNA synthesis in rat hepatocytes	Negative (PO, thus low exposure)	Negative (but low exposure since PO)
13-week oral toxicity in rats	-	NOAEL: 1000 mg/kg/day[2]
13-week dermal toxicity in rats	NOAEL: 500 mg/kg/day (47.6%)[3]	-
13-week dermal toxicity in hairless mice (2.5-20%; ± simulated sunlight)	NOAEL: 200 mg/kg/day (20%)	-
Reproduction study in rats (oral) (preliminary)	NOAEL: 1000 mg/kg/day	-
Development toxicity study in rats (oral)	-	NOEL: 1000 mg/kg/day
Androgen receptor binding assay (*in vitro*)	-	Negative
Oestrogen receptor binding assay (*in vitro*)	-	Negative
Uterotrophic assay in rats treated orally	-	Negative
Repeat dose inhalation toxicity	**No data**	**No data**
Repeat dose toxicity in non-rodent species	**No data**	**No data**
Carcinogenicity potential	**No data**	**No data**

[1] This concentration in the air (10% aqueous dilution) caused reversible pulmonary inflammation.
[2] Reversible piloerection observed at ≥250 mg/kg/day. No other effects observed at ≤1000 mg/kg/day.
[3] Scabs, vocalization and decreased mean body weight gain observed at 1000 mg/kg/day.

Introduction

The data package contained studies that were conducted under conditions of good laboratory practice (included quality assurance statements). Studies submitted included dermal absorption through skin (*in vitro* percutaneous), local tolerance (skin and eye irritation), sensitisation and photo-sensitisation, acute oral toxicity, repeat-dose oral toxicity (13 weeks), reproductive toxicity (developmental study) and *in vitro* and in vivo assays on genotoxicity, and photo-genotoxicity.

Studies submitted used tris-biphenyl triazine particle sizes of d(0.5)= 15.4 µm, 440 nm, 81 nm, 109 nm, and 120 nm. In some of these studies a comparison was made between the nanosized (≤120 nm) and non-nanosized (440-15400 nm) material. The differences in particle sizes did not produce any appreciable change of toxicity profile for tris-biphenyl triazine.
Two different batches used for studies with tris-biphenyl triazine were shown to have a similar analytical profile. The purity was 98% (w/w; expressed as active molecule). Each batch contained <0.1% of a known by-product (2-chlor-4,6-bis-(biphenyl)-triazine) and 0.32% defined as the sum of two unknown and non-coloured by-products.

Studies were submitted in which the tris-biphenyl triazine used had a particle size of d(0.5)= <15.4 µm. Since in the intended commercial product, tris-biphenyl triazine is going to be micronized to obtain particles with a d(0.5) = 100-110 nm, studies with the material to which the consumer would be exposed were also provided, in which tris-biphenyl triazine batches with a d(0.5) of between 81 and 175 nm were used. In some of these studies a comparison was made between the nanosized and non-nanosized material.

Pharmaco/toxico-kinetics

Tris-biphenyl triazine was assayed by HPLC and LC-MS and characterised by UV/Vis, IR, ^{1}HNMR and ^{13}C NMR. The particle size distribution was characterised by Scanning Electron Microscopy (SEM) and Fiber Optic Quasi Elastic Light Scattering (FOQELS).

Tris-biphenyl triazine has a high molecular weight, which would restrict significant absorption through the skin. Absorption through the skin was demonstrated in vitro for human and rat skin, with absorption rates as follows:

Assay details	Absorption	
	<175 nm	**<14500 nm**
In vitro percutaneous, rat skin	4.78%	12.77%
In vitro percutaneous, human skin	0.20%	0.57%
In vitro percutaneous, pre-damaged human skin	0.76%	-
In vivo single dermal dose in rats	-	0.11%

These values are very conservative since in several instances the drug was below the limit of quantification, and also since given a large variability in absorption values, the mean value ± 2 standard deviations were used in the calculations. In the only in vivo absorption study conducted (in rats), tris-biphenyl triazine absorption was very small, at only 0.11%.

Dermal and oral dosing in rats resulted in systemically measurable radiolabelled tris-biphenyl triazine. In the dermal absorption study, tris-biphenyl triazine plasma levels reached 200 ng/ml, and levels were not quantifiable by 48 hours. In the 13-week dermal toxicity study, tris-biphenyl triazine plasma levels reached 20 ng/ml in rats. In this study, accumulation of tris-biphenyl triazine cannot be excluded. Given the very low levels (around the limit of quantification), and the exaggerated exposure scenario, this is not considered as a concern for tris-biphenyl triazine.

Two *in vivo* ADE studies were carried out in rats dosed with tris-biphenyl triazine, one with a particle size of 87 nm, the other of 6 µm. The study with the 6 µm radiolabelled particles showed a higher absorption percentage of radioactivity, and higher levels of radioactivity in various tissues. However, the observed absorption was low. In the study using nanosized particles, about 0.06% of the dose was recovered in urine, and only the remaining carcass showed tris-biphenyl triazine above the LOQ (levels in carcass were about 0.07% of the applied dose). From the results presented, accumulation cannot completely be excluded, since half life time seems to be longer than 24 h. Also in the 13 week dermal study, an increase in blood levels was observed over time. It is not clear in which organs the particles accumulate since not all organs were analysed for residues. For the larger particles (6 µm) 0.73% of the dose was recovered in the urine. Radioactivity was recovered in a number of organs at low levels, however only levels in fat (0.01% of the dose) and the remaining carcass (0.25% of the dose) could be reliably quantified.

After oral administration of radiolabelled tris-biphenyl triazine, only a very low amount of radioactivity was absorbed from the gastrointestinal tract and almost the complete dose was excreted unabsorbed as unchanged parent with the faeces. Since dermal, oral, intraperitoneal, as well as genotoxicity studies, were performed in vivo, the safety of the potential metabolites have been established in the nonclinical species. After topical application, only a very small fraction of tris-biphenyl triazine is absorbed. Therefore the potential systemic consumer exposure and therefore the exposure to potential metabolites is expected to be very low.

As can be seen, there was no increase in dermal absorption with particles of smaller size. This was also true of absorption after oral dosing in rats (0.06% of the dose was absorbed when smaller particles were used, cf 0.73% for bigger particles). The oral absorption studies demonstrated that the oral toxicity study results are valid, since the animals were exposed to absorbed tris-biphenyl triazine.

Calculation of safety margin of tris-biphenyl triazine by the SCCS is present in Appendix 1. The safety margins were recalculated by the nonclinical evaluator and they are discussed in the specific sections of dermal exposure and oral exposure.

Interaction with other UV filters

There were no data on the possible interaction of tris-biphenyl triazine with other UV filters likely to be used to formulate sunscreen products. Information on the stability of tris-biphenyl triazine showed no variation in the substance (purity: ≥97% in all instances; no significant changes in appearance, degradation products, colour, assay, odour, IR spectrum) under conditions of changing storage tests at different temperature

and humidity over periods of 6 months (40°C/75% RH) and 24 months (25°C/60% RH). No significant differences of active content of FAT 65'080 were found without irradiation and after irradiation with 3 MED (minimal erythemal dose) of the sunscreen formulation. A comparative Absorbance spectra for a sample after 8-months storage at room temperature and for a newly prepared formulation showed the shape of each UV absorption spectrum to be similar.

It would be possible that tris-biphenyl triazine is formulated as a sole UV filter in a product since it appears to be a broad-spectrum UV filter that provides protection from UVB and UVAII wavelengths from 290 to 340 nm (see UV-spectrum below). Lack of specific interaction data with other UV filters could be justified by the stable (and photostable) nature of tris-biphenyl triazine.

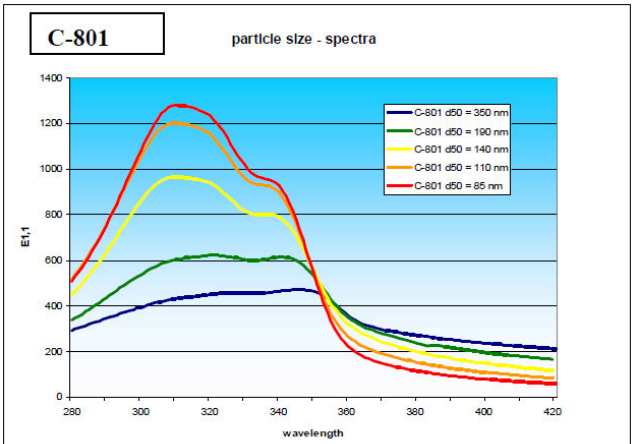
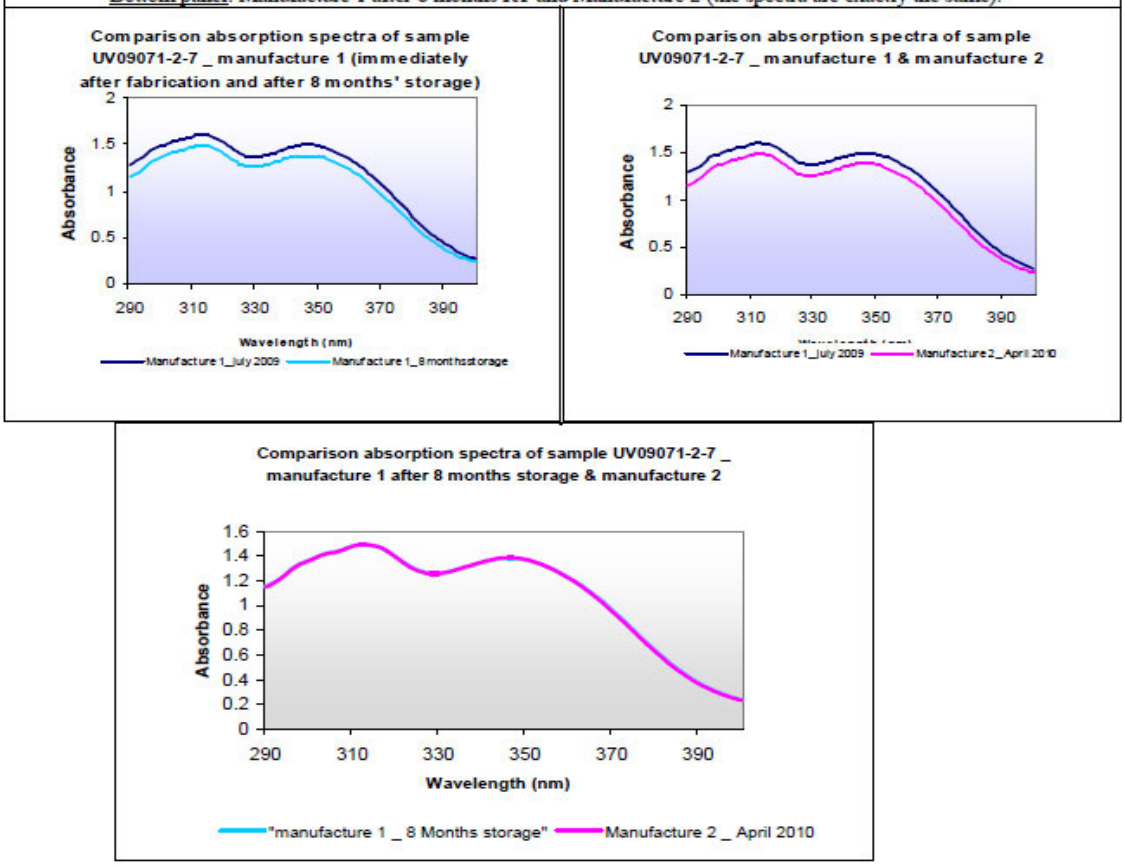


Figure 1.
- Maximum E1,1 increases with decreasing particle size
- Maximum area under the curve is reached at 120 nm

Stability

A representative commercial formulation (oil-in-water type) was prepared and measured at time 0 and after 8 months storage at room temperature. The Sponsor stated that the abosrbance spectrum at time 0 was conducted without recording the exact amount of formulation applied to the PMMA plates used for the test. The absorbance spectrum remains representative and will represent the absorbance spectrum for the different particle sizes.

Figure 3. Comparison of UV Absorbance for SPF 50 Commercial Sunscreen formulation.
Left panel: Manufacture 1 (2009) formulation before and after 8-months storage at room temperature.
Right panel: newly made formulation (Manufacture 2) immediately after preparation.
Bottom panel: Manufacture 1 after 8 months RT and Manufacture 2 (the spectra are exactly the same).

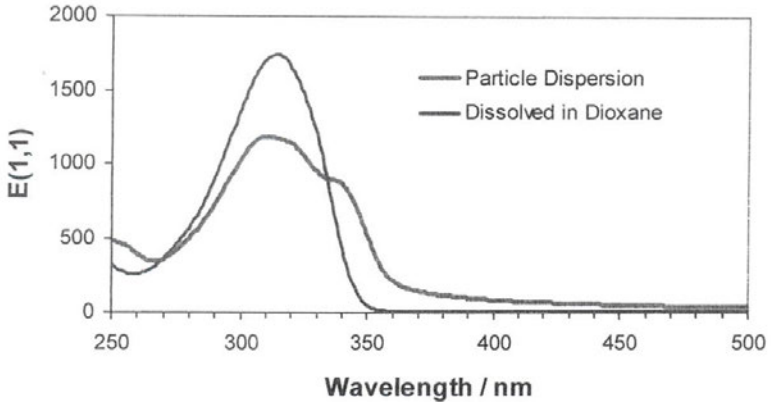


Stability and homogeneity of dosage forms of 10, 50 and 200 mg/mL was measred and found to be satisfactory in the batch used (purity 98%) in the 13-week oral toxicity study in rats. Nominal and measured concentrations of tris-biphenyl triazine for each administered dosage form analysed was found to differ by less than 9%.

Stability of tris-biphenyl triazine in the cream formulation used in the human studies was checked by HPLC-DAD. No significant differences within active content of tris-biphenyl triazine were found without irradiation and after irradiation with 3MED (minimal erythemal doses) of the sunscreen formulation.

Photostability

The UV-spectra of the active ingredient were measured with the substance dissolved in dioxane and then also with particles of the substance with size of 105 nm dispersed in water. Below is the UV/Vis Spectra of tris-biphenyl triazine in solution and in dispersion in terms of the specific extinction E(1,1). The spectrum of the dispersion shows a shoulder at about 340 nm.



For photostability assessment 2 mg/cm2 of an o/w emulsion containing 2% of tris-biphenyl triazine particles were spread on roughened quartz plates. the samples were irradiated using an atlas CPS and a solar simulator. The temperature of the samples was kept between 35 and 45oC. The CPS and device were operated at 760 W/m2 total intensity. After irradiation, the samples were analyzed quantitatively for the parent substance using HPLC.

After 4 hours of irradiation, corresponding to a dose of 20 MED, there was no significant loss of the parent substance.

Local tolerance

All sensitization studies in animals (rabbit, mouse and guinea pig) were performed with tris-biphenyl triazine particles of <15 µm.

Tris-biphenyl triazine was classified as a non-irritating agent to the rabbit skin and slightly irritating to rabbit eyes after a single application.

In a murine local lymph node assay, comparison with relevant control indicated that tris-biphenyl triazine did not induce delayed contact hypersensitivity in mice at a concentration of 10%.

In sensitisation and photosensitisation studies in guinea pigs, tris-biphenyl triazine did not show any sensitisation or photosensitisation response after induction with topical application of 10%. Under the experimental conditions, two very specific wavelengths of UV radiation -UV A (365 nm) or UV B (312 nm)- were used. A wider broadband UVA and UVB irradiation would have been more informative since the absorption spectrum of the substance is expected to be 290 to 340 nm. Nevertheless, the results showed no evidence of phototoxicity or photoallergenicity at the proposed concentration of 10%.

In a human study in which 53 subjects were exposed (six cycles of application and irradiation) to a cream formulation containing 9.9% tris-biphenyl triazine (particle size of ~90 nm) and were irradiated with UV light, phototoxicity or photosensitization responses did not occur.

The undiluted tris-biphenyl triazine is likely to be an eye irritant but at the intended concentration of 10% in topical products, eye irritation may not occur. An absence of positive reaction in the guinea pig, mice and human studies is reassuring that sensitization is unlikely with tris-biphenyl triazine of particles between 100 and 15000 nm diameter.

Systemic effects after dermal application

In an acute dermal toxicity study in rats, administration of tris-biphenyl triazine (particle size <15 µm) at 2000 mg/kg (under semi-occlusive dressing on ~10% of total body surface) caused no signs of toxicity or mortality.

Dermal toxicity studies of 13 weeks duration and using nano-sized particles (81-109 nm) were performed in mice and rats.

In the hairless mice study, the animals were exposed to dose levels of up to 650 mg tris-biphenyl triazine/kg bw/day (concentration of up to 20%) and were daily exposed to UV light. No tris-biphenyl triazine-related effects on body weight, or increase in oedema formation, wrinkling, or skin fold thickness were observed, compared to control animals at this dose level and concentration.

In the rat study, the animals were exposed to dose levels of up to 1000 mg tris-biphenyl triazine/kg bw/day (concentrationS of up to 40%). The highest dose used (1000 mg/kg) caused a decrease in body weight, and therefore the NOAEL was found to be 500 mg/kg bw/day (20% concentration) cutaneously for 13 weeks in rats. Scabs, vocalization and decreased mean body weight gain were observed at 1000 mg/kg/day.

Tris-biphenyl triazine was detected in the blood of all treated groups, indicating non-dose-dependent dermal and/or oral (licking) absorption (low and variable). Since low levels were still present 2 weeks after the end of exposure, the possibility exists for accumulation of tris-biphenyl triazine.

At a concentration of 20%, tris-biphenyl triazine is well tolerated when applied dermally, with no systemic effects observed. Although the detection in the blood of treated rats may have been due to oral ingestion, accumulation of tris-biphenyl triazine is possible as suggested by the presence of low levels of the substance after 2 weeks of recovery.

It would have been advisable to have performed a repeat-dose toxicity study in a non-rodent species. However, due to the lack of significant toxicity in the rodent repeat-dose toxicity studies, as well as no local tolerance effects in humans (see ‘local tolerance above’), this deficiency is not critical.

Margin of exposure if absorbed through the skin

The following is a calculation of a margin of exposure for systemic effects provided by the Sponsor:

Comparative MoS calculations for ETH50
[Based on human skin in vitro study results for micronized
(80 nm) particle size on Normal skin]

Parameter		% of Applied
Adult Body weight (Kg)		60
Body surface area (cm^2)		17.500
Sunscreen applied (g) (if at 1 mg/cm^2)		18
FAT 65080 applied (mg) (10% formulation)		1800
Absorption function		0.06% of applied
Duration of exposure		1 day
Systemic Estimated Dose (mg/kg/day)		0.018
NOAEL (mg/kg/day) Rat 13-wk oral study		1000
Normal skin: MoS (NOAEL/SED)		**55,555**
80 nm median Particle diameter		
Damaged skin	Absorption	0.8% of applied
	MoS	**4166**
6 µm median particle diameter		
Normal skin	Absorption	0.2% of applied
	MoS	**1667**

The calculations were repeated by the TGA nonclinical evaluator using the NOAEL from the dermal study, since using the NOAEL from the oral study and not taking into account that only a small percentage of the dose was absorbed in the rats is misleading. Parameters and values used for the calculations were as follows:

- For human: 18000 mg sunscreen applied to a 50 kg person (360 mg/kg sunscreen), UV filter concentration of 10%,and a rate of dermal absorption of 0.76% in pre-damaged skin (and not 0.06% as the Sponsor used since this is an oral value), for a final dose of 0.27 mg/kg or 9 mg/m2 tris-biphenyl triazine absorbed.
- For rat: 500 mg/kg dermally (NOAEL), 4.78% rate of dermal absorption, for a total absorbed dose of tris-biphenyl triazine of 23.9 mg/kg or 143.4 mg/m2.

This results in a **ratio of rat-to-human** absorbed dose at the NOAEL of **88** (based on mg/kg) or **16** (based on mg/m2). The ratios are higher if the absorption rate value for non-damaged human skin (0.57%) is used: **117** (based on mg/kg) or **21** (based on mg/m2).

These ratios are acceptable in light of the lack of appreciable toxic effects in all of the studies submitted (except for decreased body weight in rats receiving 40% tris-biphenyl triazine dermally at 1000 mg/kg/day and hypoactivity and piloerection in mice

receiving ≥1000 mg/kg in the micronucleus assay and rats at ≥250 mg/kg/day po in the 13-week study. Furthermore, the calculation of the absorption values were very conservative since most of the measured values were below the limit of quantification.

Oral toxicity (acute/repeat dosing)

Acute oral toxicity studies in rats showed that tris-biphenyl triazine was of low acute toxicity (>1 and >2 g/kg for nano-sized and micro-sized particles respectively). In these studies, there were no clinical signs of toxicity, no departure from normal body weight development, no mortalities and no unusual lesions at necropsy. The number of animals used in the study (limit test; 3/sex) was lower than the number conventionally used (5/sex; OECD guidelines) but for an acute oral toxicity study, the low number is not considered a major deficiency.

No signs of systemic toxicity were observed when tris-biphenyl triazine was evaluated orally with repeat dosing (for 13 weeks) and in a reproductive and developmental studies, all in the rat and all at up to 1000 mg/kg/day. In the 13-week oral toxicity study, rats receiving tris-biphenyl triazine at up to 1000 mg/kg/day displayed piloerection and slight haematological changes that could not definitely been attributed to the treatment and which were not significant. The extent of oral bioavailability in the rat studies was ascertained to be very low.

Reproductive toxicity/hormonal activity

In a series of studies, potential adverse effects of tris-biphenyl triazine on the reproductive function were evaluated.

In a preliminary one-generation reproductive and developmental toxicity study (with a low number of animals), no adverse systemic effects, maternal or developmental toxicity, were observed in rats receiving 1000 mg tris-biphenyl triazine/kg bw/day (with a particle size of 109 nm).

Since tris-biphenyl triazine did not displace 3H-R1881 from cytosolic preparations of rat prostate gland tissue or 3H-Estradiol from cytosolic preparations of rat uterine tissue, it was concluded that tris-biphenyl triazine did not possess intrinsic potential to interact with the rat androgen or oestrogen receptor in in vitro receptor binding assays.

Direct measurement of hormone levels to determine whether a change was induced by tris-biphenyl triazine was performed in the oral 13-week study. Although some changes were observed, they were determined to be due to oestrus cycles and were not biologically or toxicologically significant.

Organ weight (defacto rat uterotrophic assay) analysis did not show any increase in the size of the rat uterus in a study in which rats received tris-biphenyl triazine at up to 1000 mg/kg/day for 3 days orally.

Developmental studies in rats treated with tris-biphenyl triazine (up to 1000 mg/kg/day PO) did not show any apparent adverse effects/abnormalities in foetuses. It was also apparent that there were no adverse effects on reproductive parameters during the course of the studies.

A NOEL of 1000 mg/kg/day PO was determined for reproductive effects (including developmental effects) of tris-biphenyl triazine in a preliminary study in rats.

Genotoxicity

Genotoxicity was examined *in vitro* in bacterial and mammalian cell systems, as well as *in vivo* in rats and mice. All in vitro genotoxicity studies were performed with the bigger particles of tris-biphenyl triazine (<15 µm), whereas both in vivo studies were performed with the bigger and the smaller particles (~80 nm).

In vitro testing included reverse mutation assays in S. typhimurium and E. coli, mouse thymidine kinase locus gene mutation assay, chromosomal aberration assays in Chinese hamster V79 cells (including irradiation) and in cultured human lymphocytes, and a photo-mutagenicity assay in E. coli (reverse mutation). In all these tests there was no evidence that tris-biphenyl triazine was genotoxic.

The validity of the in vitro tests could be questioned on the grounds that tris-biphenyl triazine (MW 537.7) may not have been able to penetrate cells to interact with genetic material. However, in vivo studies were also conducted. An unscheduled DNA synthesis test in rat hepatocytes using tris-biphenyl triazine at particle sizes of <15.4 µm and 81 nm, did not show genotoxicity. Given the low oral absorption and dosing via gavage, it is not clear whether in the UDS test the cells were adequately exposed. However, an in vivo mouse bone marrow assay was also negative, and in this study optimal exposure would have been achieved. It is worth noting that in these mice, the only clinical findings were piloerection and hypoactivity at 1000 (81 nm particles) and 2000 (15 µm particles) mg tris-biphenyl triazine/kg intraperitoneally.

Since the in vitro studies were conducted in the presence of metabolic activation, and since the genotoxicity studies in vivo were negative, the potential for the formation of active metabolites has been addressed satisfactorily. Overall, the data indicate no concern with regard to potential genotoxicity of tris-biphenyl triazine.

Carcinogenicity

No carcinogenicity study was submitted. Generally recognised criteria for the rationale to perform carcinogenicity studies on a substance include large human systemic exposure to the substance, long duration of human exposure, structural alert features of the substance, genetoxicity or clastogenicity, adverse findings in in vivo repeated-dose toxicity studies (immune modulation, hormonal modulation and non-neoplastic cell changes) and toxicokinetic studies showing formation of active metabolite/s (stored in tissues).

In case of tris-biphenyl triazine, the likely systemic exposure would be low since the products containing tris-biphenyl triazine are for dermal application and tris-biphenyl triazine is poorly absorbed through the skin (0.2-0.6% in human skin in vitro). Therefore, the expected systemic of tris-biphenyl triazine is very low limiting the overall burden on the body.

No features of tris-biphenyl triazine suggest potential carcinogenicity activity of the molecule.

Since tris-biphenyl triazine was negative in all the genotoxicity studies performed (both in vitro and in vivo), it is unlikely that tris-biphenyl triazine is a carcinogen. tris-biphenyl triazine was non-sensitizing, confirming the non-reactive nature of the molecule to biological molecules.

There was no evidence of non-neoplastic changes associated with tris-biphenyl triazine given by either the oral or dermal routes to rats for 13 weeks. There were no indications of a potential target organ or effects on reproductive organs that may be suggestive of an interaction with hormonal activity.

A non-genotoxic mechanism for carcinogenesis involving chronic skin irritation and inflammation could be relevant for topically applied substances such as UV filters in sunscreens. However, data are available from skin irritation studies and repeat dose dermal toxicity, which indicate tris-biphenyl triazine was not a primary skin irritant.

Overall, the data provided by the Sponsor adequately justifies the lack of a dedicated carcinogenicity study.

Inhalation toxicity

In one acute inhalation study, and using only one dose level presented using tris-biphenyl triazine (particle size of 109 nm), no lethality was observed in rats. However, a strong inflammatory response was seen in the lung of exposed animals after 4 hours, and this was not completely resolved after 15 days.

The following is a calculation provided by the Sponsor, of a margin of exposure for the inhalation effects observed in rats:

Relevance to human exposures via spray-on sunscreen formulations.
While the rat inhalation study with ETH50 in aerosol did show a reversible lung inflammatory response in the rat study, it must be considered that this was an extreme exposure situation that, like the Landsiedel et al study, could better represent potential workplace or industrial situations.

For our purposes, we will show how the rat inhalation study compares to the estimated human exposure that could result from use of spray-on sunscreen formulations. We make the following assumptions and calculations:

1. Application at 1 mg sunscreen/cm^2 to skin (17,500 cm^2) = 18 g/application
2. ETH50 present in sunscreen at 10% in aerosol or pump spray formulation
3. Application of 1800 mg a.i. twice in one day
4. Exposure during application is about 10 minutes (0.17 h)
5. Volume of air around the application zone ca. 4 m^3 (rough estimation)
6. Human ventilation rate 0.83 m^3 per hour; body weight 60 kg

Inhalation exposure: = 126 mg ETH50 or
2.1 mg/kg bwt/d

([1800 mg/ 4 m^3] x [0.83 m^3/hr] x [0.17 hr] x 2 applications)/ 60 kg

7. Commercially available spray applicators deliver droplets of about 80 µm diameter if formulation contains 4% of insoluble type UV Filters (Durand et al. 2007)[7]; about a 10% will be diameter 10 µm or less.
8. 10% is respirable size = 0.2 mg/kg bwt/d

Margin of Exposure: 20,000
rat inhalation vs human inhalation: [4000 mg/kg/d] / 0.2 mg/kg/d]

The calculations were repeated by the TGA nonclinical evaluator, using more conservative values, i.e. 1 m^3 as the volume of air around the application zone (instead of 4), 50 kg body weight (instead of 60), and 100% respirable size (instead of 10%).
The result was a **margin of exposure of 394** ([4000 mg/kg/day] / [10.16 mg/kg/day]).
This margin of exposure is still sufficiently high, since

- The values used for the calculations are very conservative.
- The lung inflammation observed was reversible (recovery not complete by 15 days in severe cases).
- The rat experiment used the maximally achievable aerosol concentration of tris-biphenyl triazine.
- In the rat experiment, the mass median aerodynamic diameter of tris-biphenyl triazine was 1.2 µm. In contrast, in pump sprays and propellant sprays, the mean diameter was >20 µm (Study of effects of spray type -propellant or pump spray- on Droplet/particle size). The droplet fraction below 10 µm was below 10% (propellant sprays, butane/propane) and 1% (pump sprays). Particles of >20 µm diameter which will presumably be delivered by sprays will not reach the lung so readily. Most particles >10 µm are deposited in the oropharyngeal region with a large amount impacting on the larynx, and aerosols with a mass median aerodynamic diameter of 5–10 µm are mainly deposited in the large conducting airways and oropharyngeal region[4]. Even with drugs that are formulated to be inhaled, and using a metered dose inhaler, only 10–20% of the emitted dose is deposited in the lung, due to 50–80% of the drug aerosol impacting in the oropharyngeal region and also to problems co-ordinating actuation of the device with inhalation[5].

However, the safety of repeated use of tris-biphenyl triazine at 10% in sprays cannot be ascertained, due to:

- uncertainties regarding how the nano-material would be cleared from the lung;
- the lack of repeat-dose inhalation toxicity studies,
- the lack of an identified NOEL for inhaled dosing.

The marked increase in neutrophils and macrophages in the lung (some of the pulmonary effects observed had not returned to normal after 2 weeks) suggest the presence of a strong inflammatory response.

[4] Labiris NR, Dolovich MB. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol. 2003 Dec; 56(6): 588–599.
[5] Labiris NR, Dolovich MB. Pulmonary drug delivery. Part II: The role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol. 2003 Dec;56(6):600-12.

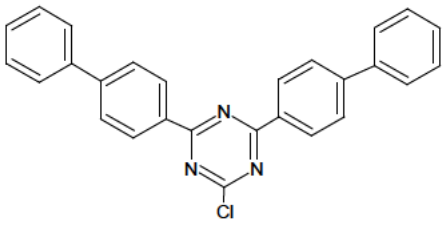
MAIN BODY

Introduction

Tris-biphenyl triazine is an active ingredient which is a broadband UV filter intended to protects the skin against UV wavelengths ranging from 290 to 340 nanometers (UVB and UVAII radiation), when used at a proposed final concentration of 10% in sunscreens.

The filter is photostable. The sponsor intends to use tris-biphenyl triazine at up to 10% in sunscreen products alone or in combination with other UV filters. The formulation will include nano-sized particles, of around 100 nm.

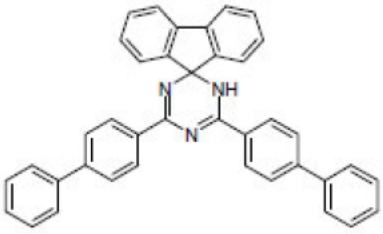
Studies submitted used materials of particle sizes of d(0.5)= 15.4 µm, 440 nm, 81 nm, 109 nm, and 120 nm. In some of these studies a comparison was made between the nanosized (≤120 nm) and non-nanosized (440-15400 nm) material. The Sponsor has stated that the small particle size is important to the Ultraviolet light absorbing efficacy of the active ingredient, and on the process used to prepare the end product to be placed on the market. All appropriate safety studies were performed under Good Laboratory Practice (GLP). The applicant stated that the purity was measured in 6 different batches and varied from 97.2% to 98.5%. The main known impurity was present at 0.1% and is designated as ETH 50/129B or 2-chlor-4,6-bis-(biphenyl)-triazine.



Impurity ETH 50/129B

In addition, 0.32% of the substance is composed of the sum of two unknown and non-coloured by-products. The balance of the composition comprised water, chloride, aluminium and residual solvents.

A by-product was found to be formed during the synthesis process. This by product was part of each batch of tris-biphenyl triazine (at concentrations of 0.003-0.025% of tris-biphenyl triazine) used for toxicological profiling:



Molecular Weight =537.67
Exact Mass =537.22
Molecular Formula =C39H27N3

4',6'-Bis-biphenyl-4''-yl-1'2'-dihydro-spiro[9H-fluorene-9,2'[1,3,5]triazine]; By-product of tris-biphenyl triazine

Since the identified impurity and by-product are structurally related to the active UV filter, all impurities were present in batches used to demonstrate the safety profile of the proposed UV filter, and the absorption of impurities is expected to be negligible. Their safety profile has been stablished.

International regulatory status

Tris-biphenyl triazine is a new UV-A2 and UV-B filter (absorbance max. ~340 nm) for use in listed sunscreen products. It was reviewed by the Scientific Committee on Consumer Safety (SCCS/1429/11) in September 2011 (the first submission was received in 2005), and was **included in the positive list (Annex VI)** of the EU Cosmetics Regulation (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32014R0866) with the following conditions: 1) not to be used in sprays, 2) nanomaterials with median primary particle size of >80 nm, purity of ≥98% and uncoated are allowed, and 3) a maximum concentration (in ready for use preparation) of 10%.

The applicant provided assurance that Tinosob A2B/ Tris-biphenyl triazine

- does not appear in Annex II to the EEC Directive 76/768 list of substances which must not form part of the composition of cosmetic products;
- was listed in Annex VI of the European Cosmetics Regulation on 8 August 1014. (Refer to Official Journal of the European Union L238/3 http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2014.238.01.0003.01.ENG);

International regulatory comment

The applicant has stated that the name of the active is included in the International Cosmetic Ingredient Dictionary. Included in the data package was a copy the Opinion of the Scientific Committee on Consumer Safety (SCCS) (Scientific Committee on Consumer Safety), Opinion on 1,3,5-
triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-, 20 September 2011

The SCCS from the European Commission concluded that:

- Dermal exposure to formulations containing the UV-filter tris-biphenyl triazine with a mean particle size of 81 nm, as described in the dossier, results in low absorption of tris-biphenyl triazine. Also after oral exposure, absorption of tris-biphenyl triazine is low. No systemic effects are observed after oral or dermal exposure up to 500 mg/kg bw/day. Due to the low bioavailability of tris-biphenyl triazine, a risk assessment based on a NOAEL from oral studies and applying route-to-route extrapolation was not considered appropriate. Based on comparison of the internal dose in man and rat (resulting in a MoE of 357) and comparison of the NOAEL in the 13 week dermal study in the rat and the human systemic exposure dose it was concluded that the use of 10% tris-biphenyl triazine can be considered safe for dermal application.

- The risk assessment of nanomaterials is evolving. It should be noted that the testing of the substance and the present assessment are based on methodologies initially developed for toxicity testing of substances in non-nano form and current knowledge. From this perspective it is concluded that the use of 10% tris-biphenyl triazine can be considered safe for dermal application. This assessment, however, is not intended to provide a blue-print for future assessments, where depending on the developments in methodology and risk assessment approaches and probable development of nano-specific testing requirements, additional/different data could be required and/or requested on a case-by-case basis.

Regarding restrictions to the safe use of 1,3,5-Triazine, 2,4,6- tris[1,1'-biphenyl]-4-yl-, the SCCS concluded:

- At this moment there is too much uncertainty to conclude about safe use of 10% tris-biphenyl triazine in spray applications, because of concerns over possible inhalation exposure. Therefore, the SCCS concludes that spray products containing ETH-50 cannot be recommended until additional information on safety after repeated inhalation is provided.

The nonclinical evaluator of the TGA agrees with the conclusions of the SCCS regarding the low absorption of tris-biphenyl triazine after dermal exposure to formulations containing tris-biphenyl triazine (including with a mean particle size of 80-100 nm results), as well as after oral exposure. No systemic effects were observed after oral or dermal exposure up to 500 mg/kg bw/day. The margin of exposures were recalculated by the nonclinical evaluator using more conservative values, and they were still satisfactory. The use of tris-biphenyl triazine at concentrations of up to 10% in sunscreen formulations for use in non-damaged skin is supported by the data.

Adverse effects

The sponsor did not provide information on reports of adverse events.

Product specifications

The following product specifications were provided:

Product specification

Tinosorb® A2B

PRD 30478125

Care Chemicals

Characteristic values

The specifications stated in the paragraphs 'Quality control data' and 'Additional product descriptive data' finally and conclusively describe the properties of the product.

Quality control data

(Data which is used for quality release and is certified for each batch.)

Test property	Specification	Test method
Appearance	Viscous dispersion	Visual test
Product color	White to distinctly beige dispersion	Visual test
pH	10.5 - 12.0	pH determinant glas electrode
Absorbance (5 mg/l, TMU, Mesitylene, Dioxane, 316 nm, 10 mm)	0.442 - 0.498	UV-VIS spectroscopy
Absorbtion (1 % dil./1 cm)	885 - 998	UV-VIS spectroscopy
Active substance	47 - 53 %	UV-VIS spectroscopy
Viscosity (25 °C; Haake/MVDIN; D = 100 1/s)	200 - 1200 mPas	DIN 53019
Dry content (1 g / 135 °C / 60 minutes)	55. 0 - 62.0 %	IR drying
Total Viable Count	<= 100 CFU/g	Test method CG 184

Notes:

Manufacturer: BASF Grenzach GmbH, Köchlinstr.1,DE-79639 Grenzach-Wyhlen, Tel.: +49(0)7624 120

Responsible for release: Dr. Elke Polley, Quality Unit Grenzach.

Storage information

Shelf life

24 months

Storage conditions

In original sealed containers and protected from moisture.

SUMMARY OF SUBMITTED TOXICITY DATA

Tris-biphenyl triazine was reviewed by the European Scientific Committee on Consumer Safety (SCCS/1429/11) in September 2011. The data package included a copy of the Opinion of the Scientific Committee on Consumer Safety (SCCS) (Scientific Committee on Consumer Safety), Opinion on 1,3,5-triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-, 20 September 2011. Only the studies provided to Australia are summarised below. For further information on study details, the reader is referred to the SCCS opinion on the UV Filter (Appendix 1), the Sponsor's Overview of information (Appendix 2) and document of communications between the Sponsor and the European Agency (Appendix 3).

Summary of study details

Study details. Particle size	Additional information	Conclusions
Toxicokinetics		
Study no.: A00112. Percutaneous Penetration, rat and human skin membranes. Particle size: 440 nm. Description of the study found on page 52 of Appendix 1.	RCC Ltd, CH-4452 Itingen, Study number A00112 (03 August 2005). [14C] FAT 65080 (tris-biphenyl triazine) Percutaneous Penetration of Micronized [14C] FAT 65080 (tris-biphenyl triazine) Through Rat and Human Split-thickness Skin Membranes (in-vitro)	Micronized FAT 65080 (440 nm; 2 mg/cm^2 in 13 µL for 24 hours), applied to rat and human skin membranes, penetrated at an extremely low rate and to a very limited extent through the skin membranes. The penetration through rat split-thickness skin membranes was slightly higher than through human split-thickness skin membranes. The penetration into lower skin layers (below stratum corneum) was more pronounced in rat skin membrane. The dermal in vitro absorption value (given the large variation in the data the value is set as the mean absorption + 2SDs) for the rat was 12.77%, and for humans 0.57% (these are very conservative values, since they are driven by a large dose remaining on the skin membrane).
Study no.: B23624. Percutaneous Penetration. Particle size: 86 nm. Description of the study found on page 17 of Appendix 1.	RCC 2007. [14C] FAT 65080 (tris-biphenyl triazine) Percutaneous Penetration of [14C] FAT 65080 (tris-biphenyl triazine), Micronized According to Technical Specifications, Through Rat	The test item (86 nm; 2 mg/cm^2 in 13 µL for 24 hours), did not penetrate through the skin membranes to a significant extent. The dermal in vitro absorption value (given the large variation in the data the value is set as the mean absorption + 2SDs) for the

Study details. Particle size	Additional information	Conclusions
	and Human Split-thickness Skin Membranes (in-vitro). RCC study no. B23624; 1 November 2007.	rat was 4.78%, and for humans 0.2% (these are very conservative values, since most of the measured values were below the LQ). Absorption was lower for the smaller particles (cf. Study no. A00112).
Study no.: A22432. Dermal absorption rat. Particle size: 440 nm. Description of the study found on page 54 of Appendix 1.	RCC 2005. Dermal Absorption of Micronized [14C] FAT 65080 (tris-biphenyl triazine) in the Rat (in vivo). RCC study no. A22432.	Very low absorption dermally (0.11%). The systemically absorbed test item was slowly excreted in the urine and the faeces. Within 72 hours 0.05% and 0.03% of the dose were excreted in the urine and faeces, respectively.
Study no.: B23613. ADME of [14C] FAT 65080 (ETH 50) in rats. Particle size: 86 nm. Description of the study found on page 26 of Appendix 1.	RCC 2007. [14C] FAT 65080 (ETH 50): Absorption, Distribution and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. B23613; 1 November 2007.	After oral administration of [14C] FAT 65080 only a very small amount of radioactivity was absorbed from the gastrointestinal tract (0.06%) and almost the complete dose was excreted unabsorbed as unchanged parent in the faeces.
Study no.: A89280. ADME of [14C] FAT 65080 (ETH 50) in rats. Particle size: 6000 nm. Description of the study found on page 28 of Appendix 1.	RCC 2007. [14C] FAT 65080 (ETH 50): Absorption, Distribution, Metabolism and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. A89280; 4 July 2007.	After oral administration of [14C] FAT 65080 only a very small amount of radioactivity was absorbed from the gastrointestinal tract (0.73%) and almost the complete dose was excreted unabsorbed as unchanged parent in the faeces.
Study no.: C08835. Skin penetration in vitro human, pre-damaged skin. Particle size: 120 nm. Description of the study found on page 19 of Appendix 1.	RCC 2008. [14C] FAT 65080 (tris-biphenyl triazine): Percutaneous Penetration of [14C] FAT 65080 (tris-biphenyl triazine), Micronized According to Technical Specifications, Through Pre-damaged Human Split-thickness Skin Membranes (in-vitro). RCC study number C08835; 29 October 2008.	Very low absorption and percutaneous penetration (most of the values were below limits of quantification). The test item with particle mean diameter of 120 nm did not penetrate through the skin membranes to a significant extent (only 0.76%) and the damaged stratum corneum did not result in a significantly increased penetration rate of the nanosized test item.

Study details. Particle size	Additional information	Conclusions
Acute Tox Oral/Dermal/ Inhalation		
Study no.: 32808. Acute oral toxicity in rats. Particle size: 81 nm. Description of the study found on page 14 of Appendix 1.	CIT 2007. Acute oral toxicity in rats, acute toxic class method. CIT study no. 32808 TAR; 11 September 2007.	LD50 >1000 mg/kg (F rats) PO
Study no.: 26394. Acute oral toxicity in rats. Particle size: 15000 nm. Description of the study found on page 49 of Appendix 1.	CIT F-Evreux, Laboratory Study number 26394 TAR (21 November 2003). Acute oral toxicity study in rats "Acute Toxic Class Method" with FAT 65'080/A	LD50 >2000 mg/kg (rats) PO. At 200 mg/kg bw, no clinical signs were observed. At 2000 mg/kg bw, **piloerection** and dyspnea, together with hypoactivity in females, were observed in all animals on day 1.
Study no.: 26395. Acute dermal toxicity in rats. Particle size: 15000 nm. Description of the study found on page 49 of Appendix 1.	CIT F-Evreux, Laboratory Study number 26395 TAR (21 November 2003). Acute dermal toxicity study in rats with Fat 65'080/A	LD50 >2000 mg/kg (rats) dermally (under semi-occlusive dressing on ~10% of total body surface). No signs of toxicity were observed.
Study no.: B38698. Acute inhalation toxicity in rats. Particle size: 109 nm. Description of the study found on page 15 of Appendix 1. Expanded at the end of this table.	RCC 2007. 4-Hour Acute Inhalation Study in Rats with FAT 65'080/F. RCC study no. B38698. 16 November 2007	**After inhalation of particles there was massive influx of neutrophils and increase of macrophages by approximately a factor of 2. Some of the strong inflammatory response of the host after inhalation of tris-biphenyl triazine remained even on day 15.**
Irritation and Sensitisation		
Study no.: 26396. Skin irritation in rabbits. Particle size: 15000 nm. Description of the study found on page 50 of Appendix 1.	CIT F-Evreux, Laboratory Study number 26396 TAL (12 November 2003). Primary dermal irritation study in rabbits with FAT 65'080/A	Under the experimental conditions, the test item was non-irritant when applied topically to rabbits (500 mg to intact skin under semi-occlusion for 4 hours).
Study no.: 26397. Ocular irritation in rabbits. Particle size: 15000 nm. Description of the study found on page 51 of Appendix 1.	CIT F-Evreux, Laboratory Study number 26397 TAL (02 December 2003). Primary eye irritation study in rabbits with FAT 65'080/A	The substance was slightly irritant to the eye of rabbits under the conditions of the test (100 mg of the test article as a powder in the conjunctival sac of the left eye; rinsing after 24h).
Study no.: 26399. Skin	CIT F-Evreux, Laboratory	Under these experimental

Study details. Particle size	Additional information	Conclusions
Sensitization Potential in Mice (LLNA). Particle size: 15000 nm. Description of the study found on page 51 of Appendix 1.	Study number 26399 TSS (16 January 2004). Evaluation of Skin Sensitization Potential in Mice using the Local Lymph Node Assay (LLNA) with FAT 65'080/A.	conditions, the test item (at 10%) did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.
Study no.: 26400. Phototoxicity and photoallergenicity in Guinea pigs. Particle size: 15000 nm. Description of the study found on page 61 of Appendix 1.	CIT F-Evreux, Laboratory Study number 26400 TSG (22 January 2004). Phototoxic and photoallergenic Potential by Cutaneous route in guinea pigs of FAT 65'080/A	No cutaneous reactions which could be attributed to a photoirritant or photoallergenic effect of the test substance (at 10%) were recorded. Under the experimental conditions, two very specific wavelengths of UV radiation -UV A (365 nm) or UV B (312 nm)- were used without information of the absorption spectra of the substance. Broadband UVA and UVB irradiation would more appropriately mimic the intended use of this UV-filter.
Study no.: SRL 2005-089. Human Phototoxicity and Photoallergenicity test. Particle size: 90 nm. Description of the study found on page 34 of Appendix 1.	Suncare Research Laboratories, LLC, Winston Salem, NC 27106USA. Study number: SRL2005-089 (October 7, 2005. Human Phototoxicity and Photoallergenicity test with FAT 65'082/B.	The test substance (at 9.9%) showed no significant potential for phototoxicity or photoallergenicity.
Genotoxicity		
Study no.: 26390 MMT. Ames test. Particle size: 15400 nm. Description of the study found on page 57 of Appendix 1.	CIT F-Evreux, Laboratory Study number 26390 MMT (30 December 2003). Bacterial reverse mutation test with FAT 65'080/A.	Under the conditions of the test, tris-biphenyl triazine did not show mutagenic activity in the bacterial reverse mutation test with Salmonella typhimurium and Escherichia coli.
Study no.: 28643MLH. Chromosomal Aberration Study (human lymphocytes). Particle size: 15400 nm. Description of the study found on page 57 of Appendix 1.	CIT F-Evreux, Laboratory Study number 28643 MLH (05 August 2005). In vitro mammalian chromosomal aberration test in cultured human lymphocytes with FAT 65'080/B	Under the conditions of the test, tris-biphenyl triazine did not induce chromosome aberrations in cultured human lymphocytes.

Study details. Particle size	Additional information	Conclusions
Study no.: 28644 MLY. In vitro mammalian mutation (TK locus). Particle size: 15400 nm. Description of the study found on page 59 of Appendix 1.	CIT F-Evreux, Laboratory Study number 28644 MLY (18 August 2005). In vitro mammalian cell gene mutation test in L5178 TK+/- mouse lymphoma cells	Under the experimental conditions, tris-biphenyl triazine did not show any mutagenic activity in the mouse lymphoma assay.
Study no.: 868701. Photo-mutagenicity. Particle size: 15400 nm. Description of the study found on page 62 of Appendix 1.	RCC Ltd, CH-4452 Itingen, Study number 868701 (April 01, 2005). Photomutagenicity in a Salmonella typhimurium reverse mutation assay with FAT 65'080/B	Under the conditions of the test, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Study no.: 868702. Chromosomal Aberration Test. Particle size: 15400 nm. Description of the study found on page 63 of Appendix 1.	RCC Ltd, CH-4452 Itingen, Study number 868702 (July 04, 2005). Chromosome Aberration test in vitro: Photomutagenicity in Chinese hamster V79 cells with FAT 65'080/B	The test item did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) after irradiation with artificial sunlight when tested up to precipitating concentrations.
Study no.: 32753 MAS. Bone Marrow Micronucleus Test (mice). Particle size: 81 and 15400 nm. Description of the study found on page 30 of Appendix 1.	CIT 2007. Bone Marrow Micronucleus Test by Intraperitoneal Route in Mice. CIT study no. 32753 MAS. 16 June 2008.	The test item (regardless of its particle size) did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two i.p. administrations at dose levels of 500-1000 and 2000 mg/kg bw/day. The test item in vivo is not clastogenic or aneuploidic under the conditions of the study.
Study no.: FSR 070101. Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes. Particle size: 81 and 15400 nm. Description of the study found on page 29 of Appendix 1.	CIT 2007. In vivo/in vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with FAT 65'080. Institute Pasteur de Lille report no. FSR-IPL 070101. 16 June 2008. Final report amendment number 1 dated 14 January 2009.	The test item (regardless of its particle size) was not mutagenic or genotoxic. However, given the low oral absorption, exposure by the hepatocytes cannot be demonstrated. Therefore, this study is of limited relevance.
Repeat Dose and Reproductive Developmental Toxicity		
Study no.: 28341 TCR.	CIT F-Evreux, Laboratory	**Piloerection** (1/10 animals at 250

Study details. Particle size	Additional information	Conclusions
13-week oral toxicity study in rats. Particle size: 15400. Description of the study found on page 56 of Appendix 1.	Study number 28341 TCR (07 September 2005). 13-week toxicity study by oral route (gavage) in rats followed by a 4-week treatment-free period with FAT 65'080	mg/kg and 3/16 at 1000 mg/kg/day) which disappeared after ten weeks was observed. This acute effect was also seen in the acute oral toxicity study. Slight to moderate differences in white blood cell counts and biochemistry.
Study no.: 32799RSR. Combined Repeat dose oral with Repro & Developmental tox. Particle size: 109 nm. Description of the study found on page 21 of Appendix 1.	CIT 2008. Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. CIT study no. 32799 RSR. 30 July 2008. Final report amendment number 1 dated 4 December 2008.	The nanosized test article did not show adverse effects to the systemic toxicity endpoints, mating and reproduction parameters, offspring survival to 5 days of age, or to grossly and microscopically evaluated organs and tissues. NOAEL: 1000 mg tris-biphenyl triazine/kg bw/day PO, the highest dose tested. Note: small group size of 10/sex.
Study no.: 32404TCR. 13-week dermal toxicity study in rats. Particle size: 109 nm. Description of the study found on page 23 of Appendix 1.	CIT 2008. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008.	Decrease in body weight in rats receiving 1000 mg/kg/day. NOAEL: 500 mg/kg bw/day cutaneously for 13 weeks in rats. In all dose groups tris-biphenyl triazine was detected in the blood, indicating non-dose-dependent dermal and/or oral absorption (low and variable). Low levels were still present 2 weeks after the end of exposure (week 15), suggesting the possibility of accumulation of the substance/particles.
Study no.: XAW00001. 13-week Topical Range-Finding Study Hairless Mice (± simulated sunlight). Particle size: 81 nm. Description of the study found on page 32 of Appendix 1.	CRL-2007. Thirteen-Week Topical Range-Finding Study of FAT65'080/E in Hairless Mice, With or Without Simulated Sunlight. Charles River Laboratories Preclinical Services, Horsham, PA, USA; study number XAW 00001; 13 June 2007.	The topical administration of nanosized tris-biphenyl triazine with and without UV radiation did not adversely affect oedema formation, wrinkling, or skin fold thickness (which were higher in animals not receiving the test article). Tris-biphenyl triazine improved these parameters cf. mice treated with the vehicle alone or with UVR alone, indicating the tris-biphenyl triazine formulation with 81 nm particles leads to a decreased incidence of markers of UV radiation damage **indicative of**

Study details. Particle size	Additional information	Conclusions
		an efficacious protective effect.
Endocrine effects		
Study no.: XR7473. Androgen receptor binding in vitro. Particle size: 15400 nm. Description of the study found on page 63 of Appendix 1.	Central Toxicology Laboratory, Alderley Park Macclesfield, UK- Cheshire. CTL/XR7473/Regulatory Report (15 June 2005). FAT 65'080/B: In vitro androgen receptor binding assay	The inability to displace 3H-R1881 from cytosolic preparations of rat prostate gland tissue, indicated that, at concentrations up to $5x10^{-4}$ M, the test substance does not possess intrinsic potential to interact with the rat androgen receptor in the in vitro androgen receptor binding assay.
Study no.: XR7474. Estrogen receptor binding study in vitro. Particle size: 15400 nm. Description of the study found on page 64 of Appendix 1.	Central Toxicology Laboratory, Alderley Park Macclesfield, UK- Cheshire. CTL/XR7474/Regulatory Report (15 June 2005). FAT 65'080/B: In vitro estrogen receptor binding assay	The inability to displace 3H-Estradiol from cytosolic preparations of rat uterine tissue, indicated that, at concentrations up to $5x10^{-4}$ M, the test substance does not possess intrinsic potential to interact with the rat oestrogen receptor in the in vitro oestrogen receptor binding assay.
Study no.: RR1054. Uterotrophic assay in immature female rats. Particle size: 15400 nm. Description of the study found on page 65 of Appendix 1.	Central Toxicology Laboratory, Alderley Park Macclesfield, UK- Cheshire. CTL/RR1054/Regulatory Report (10 June 2005). FAT 65'080/B: Uterotrophic assay in immature female rats	No effect on body weight or uterus weight was observed after receiving tris-biphenyl triazine at up to 1000 mg/kg/day for 3 days orally.
Teratology		
Study no.: 28343 RSR. Teratology study in rats. Particle size: 15400 nm. Description of the study found on page 60 of Appendix 1.	CIT F-Evreux, Laboratory Study number 28343 RSR (06 September 2005). Study for effects on embryo-fetal development by oral route (gavage) in rats with FAT 65'080	NOEL for both the maternal and the prenatal developmental toxicity was set at 1000 mg/kg bw/day PO.
Special investigations		
Effect of spray type on droplet size. Particle size: 124 nm. Description of the study found on page 35 of Appendix 1 and page 9 of Appendix 3. Expanded at the end of	BASF SE, 2010. Droplet sizing of sun sprays containing a new cosmetic UV filter (tris-biphenyl triazine) using a Laser Diffraction system (Malvern Mastersizer). This study evaluated the	Using concentrations of tris-biphenyl triazine of 8 and 10%, the mean droplet for all spray formulations (propellant sprays and pump sprays) was ≥50 µm. The droplet fraction below 10 µm was below 10% (propellant sprays, butane/propane) and 1% (pump

Study details. Particle size	Additional information	Conclusions
this table.	influence of different formulation types and technological parameters such as spray type (propellant or pump spray) or propellant gas concentration (30-40 % butane/propane) on the size and size distribution of droplets in a sunscreen emulsion containing nanosized tris-biphenyl triazine as the only UV filter in the formulation.	sprays), respectively.

Inhalation study; RCC study 38698.

In the inhalation study submitted, Tinosorb A2B was used which contained tris-biphenyl triazine micronized to a median particle size of 109 nm in an aerosol containing 0.4976 mg tris-biphenyl triazine per litre of air. The mass median aerodynamic diameter was 1.2 µm, and the exposure (nose only) was for 4 hours. A placebo group was also treated but the aerosol did not contain tris-biphenyl triazine.
Since the tissues were not analysed for residual tris-biphenyl triazine, information on particle clearance from the lungs is not available. According to the Sponsor, the exposure to tris-biphenyl triazine in this study was at the maximally achievable aerosol concentration of tris-biphenyl triazine, amounting to 5 mg a.i./m3 or about 4000 mg a.i./kg bw.

Effects of spray type (propellant or pump spray) on Droplet/particle size. Study using a Laser Diffraction system.

This study evaluated the influence of different formulation types and technological parameters such as spray type (propellant or pump spray) or propellant gas concentration (30-40 % butane/propane) on the size and size distribution of droplets in a sunscreen emulsion containing nanosized tris-biphenyl triazine as the only UV filter in the formulation.

Two different types of fluid o/w cosmetic emulsions were prepared containing nanosized tris-biphenyl triazine (mean diameter 124 nm) at concentration of 8 and 10%, respectively. In a third formulation, only Magnesium aluminium silicate was omitted cf the 10% tris-biphenyl triazine formulation.

These formulations were incorporated in aerosol cans with gas (a blend of propane and Butane, at 30 and 40%), and 2 formulations were also incorporated into cosmetic pump spray bottles. The particle size was characterized by the mass median diameter d(0.5). The measuring distance of droplets was set at 30 cm.

Results: A propellant concentration of 40% (propane/butane) led to finer aerosols compared to a concentration of 30% propellant.

Study details. Particle size | **Additional information** | **Conclusions**

Using concentrations of tris-biphenyl triazine of 8 and 10%, the mean droplet size (d(0.5); volume distribution) for all spray formulations (propellant sprays and pump sprays) using laser diffraction methodology was ≥50 µm. The droplet fraction below 10 µm was below 10% (propellant sprays, butane/propane) and 1% (pump sprays), respectively.

Page 16-19 of Appendix 3 has additional margin of exposure calculations by the applicant.

Formulation	o/w Emulsion (GEUV10079-1-2)			o/w Emulsion GEUV10079-2-2			o/w Emulsion GEUV10079-2-3
Conc. Butane/Propane	30%	40%	0% Pump-spray	30%	40%	0% Pump spray	40%
Conc. ETH50 in formulation	8%	8%	8%	10%	10%	10%	10%
Mean Diameter (D0.5, volume distribution)	210/229 µm	204 µm	132 µm	82 / 78 µm	57 µm	68 µm	50/52 µm
Mean Diameter (D0.1, volume distribution)	110/128 µm	100 µm	53 µm	29 / 29 µm	21 µm	40 µm	16/19 µm
Max. fraction of droplets below 10 µm	<1%	<1%	<1%	< 10%	< 10%	<1%	< 10%

Appendix 1. Scientific Committee on Consumer Safety, Opinion on 1,3,5-triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-, 20 September 2011.

Scientific Committee on Consumer Safety,

Appendix 2. Corrected Supplement III to the Toxicology Summary and Safety Assessment For Eth50 (Fat 65'080) as UV Filter in Sunscreen Products.

Company Overview January 2011- Correc

Appendix 3. Answers To Questions From The "Nano" Working Group of Scientific Commission for Consumer Safety.

1101 ETH50 BASF Supplement VI 07 Jan

EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

DRAFT OPINION ON
1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 12th plenary meeting

of 20 September 2011

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members

s47F [redacted]

Contact
European Commission
Health & Consumers
Directorate D: Health Systems and Products
s22 [redacted] - Risk Assessment
Office: s22 [redacted] Brussels
s22 [redacted] @ec.europa.eu

ISSN 1831-
Doi:10.2773/
ISBN 978-92-79-
ND-

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

s47F (chairman)
(SCENIHR)
(SCENIHR)
(SCENIHR)
(SCENIRH)

(rapporteur)

External experts

s47F Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg/Munich, Germany

s47F Institut für Umwelt-medizinische Forschung (IUF), Heinrich-Heine-University, Düsseldorf, Germany

Keywords: SCCS, scientific opinion, UV-filter, 1,3,5-triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-, ETH-50, directive 76/768/ECC, CAS 31274-51-8

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 1,3,5-triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-, 20 September 2011

TABLE OF CONTENTS

1. Background

ETH 50 is a new notified substance to be used as an UV-filter in sunscreen products.

The first submission for this substance was received from the applicant in November 2005. An addendum was received in November 2006.

During review of the substance, it became apparent that it would be present in the form of nano-sized particles in the formulation to which the consumer is exposed. Therefore, further tests with this form of ETH50 were requested before the evaluation could be completed.

The present submission III provides an updated dossier including studies with the nano-sized form of ETH50.

2. Terms of reference

1. Does SCCS consider that the use of ETH50 as an UV-filter in cosmetic products in a concentration up to maximum 10.0% is safe for the consumers taken into account the scientific data provided?

2. Does SCCS have any other scientific concerns for the safe use of the new UV-filter ETH50 in finished cosmetic products?

3. OPINION

This opinion presents the safety assessment of 1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl- (trade name ETH50). Initially a set of studies were submitted in which ETH50 was used that had a particle size of d(0.5)= 15.4 µm. In some of the studies partially micronized ETH50 with a particle size of d(0.5)= 440 nm was used. However, in the intended commercial product ETH50 is further micronized to obtain particles with a d(0.5) = 100-110 nm. Upon request of the SCCS a new set of studies with the material to which the consumer would be exposed was provided in which ETH50 batches with a d(0.5)= 81, d(0.5)= 109 nm and d(0.5)=120 nm was used. In some of these studies a comparison was made between the nanosized and non-nanosized material. For this partially micronized material ETH50 with d(0.5) = 6 µm has been used.

Throughout this opinion the following terms are used to describe the different materials:
Non-micronized ETH 50: ETH50 with d(0.5) = 15.4 µm
Partially micronized (to a micron size) ETH 50: ETH50 with d(0.5) = 440 nm or 6 µm.
Fully micronized (to a nano size) ETH 50: ETH50 with d(0.5) = 81 nm, 109 or 120 nm.
The term nanosized refers to the fully micronized ETH50, whereas the term non-nanosized refers to both the non-micronized and the partially micronized ETH50.

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-

3.1.1.2 Chemical names

1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-

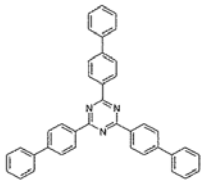
3.1.1.3 Trade names and abbreviations

ETH50
C-801
FAT 65'080
FAT 65'080/A
FAT 65'080/B
FAT 65'082/B
COLIPA n°: /

3.1.1.4 CAS / EC number

CAS: 31274-51-8
EC: not yet assigned

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_{39}H_{27}N_3$

3.1.2 Physical form

White solid

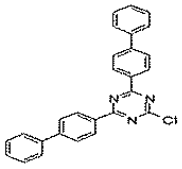
3.1.3 Molecular weight

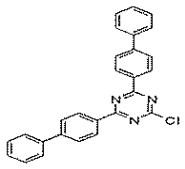
Molecular weight: 537.66 g/mol

3.1.4 Purity, composition and substance codes

Two different batches were used for studies with ETH50 and were shown to have a similar analytical profile. The purity was 98% (w/w) expressed as active molecule. In addition, each batch contained less than 0.1% of a known by-product and 0.32% defined as the sum of two unknown and non-coloured by-products; the balance of the composition comprised water, chloride, aluminium and residual solvents.
The samples were characterized by UV/VIS, IR and ^{1}H and ^{13}-C-NMR spectroscopy.

The composition of the batches used for the toxicological assays is summarised in the table below:

Batch	Measured purity active (% w/w)	Impurities	Water (%)	Others (%)
ETH 50/129B (= FAT 65'080/A)	98 ± 2	**By-product 1, 0.099 area%:** 2-chlor-4,6-bis-(biphenyl)-triazine  **Sum of 2 non-coloured & unknown by-products, 0.32 area% (overestimated value):** Structures will be similar to ETH50 and to by-product 1; they will be biphenyl-triazine derivatives as well.	0.1	<0.1 Dioxane

KoC00050/004.E (=FAT 65'080/B)	98 ± 2	**By-product 1, 0.099 area%:** 2-chlor-4,6-bis-(biphenyl)-triazine  **Sum of 2 non-coloured & unknown by-products, 0.32 area% (overestimated value):** Structures will be similar to ETH50 and to by-product 1; they will be biphenyl-triazine derivatives as well.	0.1	0.03 (xylene)

Ref.: A, B (subm I)

For the assessment of ETH, six different batches (Lots) of ETH50 have been used.
The purity was determined by HPLC with an external-standard method, and ranged from 97.2% (w/w) to 98.5% (w/w) expressed as active molecule. The by-product profile of these batches are similar and are in total <1% area. Biphenyls were not detected (detection limit < 0.01%). The content of xylene was determined by GC and ranged from 0.7% to 2%.

Ref.: A, B (subm III)

Table 1: Purity of batches used for the toxicological assays

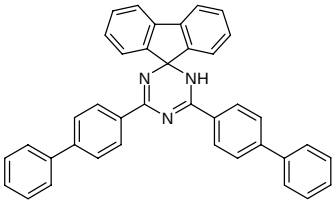
Test Substance Denomination	Batches (Lots)	Measured purity active (% w/w)
FAT 65080/D (*) ETH50= Trisbiphenyltriazin	Lot 11103Cl4AA	98.4 ± 2
	Lot 11104Cl4AA	98.5 ± 2
	Lot 11105Cl4AA	98.4 ± 2
	Lot 11106Cl4AA	98.5 ± 2
FAT 65080/E – C-801 [C-801/26 lot 5/50; KRG328-2 (Lot 04122FC7)(micronized, ETH50 (440))	Lot 11106CL4AA	98.5 ± 2
FAT 65080/F (**) (Lot 04122FC7)(micronized, ETH50 (440))	Lot 37874FC6	97.2 ± 2
	Lot 37875FC6	97.4 ± 2

(*) Mixture of 4 batches (Lots) produced within one production campaign according to the same production procedure.
(**) Active is a mixture of 2 batches (Lots), produced within one production campaign according to the same production procedure.

Analytical Profile and by-products in ETH50 (not-micronized and micronized) used for toxicity testing

A revised analytical method using LC-MS for the analysis of ETH50 in plasma samples was developed to give better detection sensitivity and as a result new information became available regarding by-products of ETH50 synthesis present in the various samples of test material used to support the safety dossier. According to the applicant, this by-product is formed during the synthesis process and has been part of each tested batch of ETH50. A recent re-analysis of the ETH50 batches has been conducted; the results for the isomer concentrations are summarized in the following Table 2.

Table 2: By-product in ETH50 used for toxicity testing

Test Substance Denominations (Batches)	**Relative Ratio** (% Isomer as ETH50 peak area)	**By-Product Found**
FAT 65080/B (KOC0050/004E)	0.008%	4',6'-Bis-biphenyl-4''-yl-1'2'-dihydro-spiro[9H-fluorene-9,2'[1,3,5]triazine]  Molecular Weight =537.67 Exact Mass =537.22 Molecular Formula =C39H27N3
FAT 65080/D (Mixture of 4 Lots: 11103CL4AA; 11104CL4AA; 11105CL4AA; 11106CL4AA)	0.025%	
FAT 65080/E KRG 238.2 Liq. (Lot 11106CL4AA micronized)	0.017%	
FAT 65080/F (Lot 04122FC7 – mixture of Lot 37874FC6 and Lot 37875FC6)	<0.003%	

Ref.: C (subm III)

Particle Size determination and Characterization

Particle size ranges for ETH50 used in studies are summarized in Table 3. In several studies the not-micronized form of ETH50 was also used in separate animal groups as a reference point or bridge to earlier study results; these results provided a comparison for evaluating if the smaller sized particles changed the study's results and outcome.

Table 3: Summary of ETH50 batches and their particle size distributions used in the additional studies submitted

FAT 65'080 Suffix Used	**Batch number**	**Particle size distribution***	**Comment**
/B	KOC00050/004.E (purity 98%)	♦ 5% < 1.3 µm , ♦ 10% < 2.4 µm ♦ 19.49% < 5 µm 50 % < 15.4 µm. (MMD)	Not micronized; used for all the studies in Annex1, except the *in vivo* and *in vitro* absorption studies.
/E	KRG328-2 (concentration 51.4% ETH50) (from LOT11106CL4AA)= MUS/KRG328-2	d(0.5) 81nm d(0.9) 157 nm	This d(0.5) is a worst case distribution compared to the d(0.5)= 100-110 nm of the commercial product's specifications.
/F	Lot 04122FC7 (concentration 47.6% ETH50) from ETH50 lot 37874FC6 & lot37875FC6	d(0.5) 109 nm d(0.9) 175 nm	This d(0.5) is within the expected range for the commercial product's specifications.

***** Particle size distribution measured by FOQELS (Fiber Optic Quasi Elastic Light Scattering)

Below is an example of a sample image. The majority of the particles in the image are below 100 nm, but also structures larger than 100 nm can be observed.

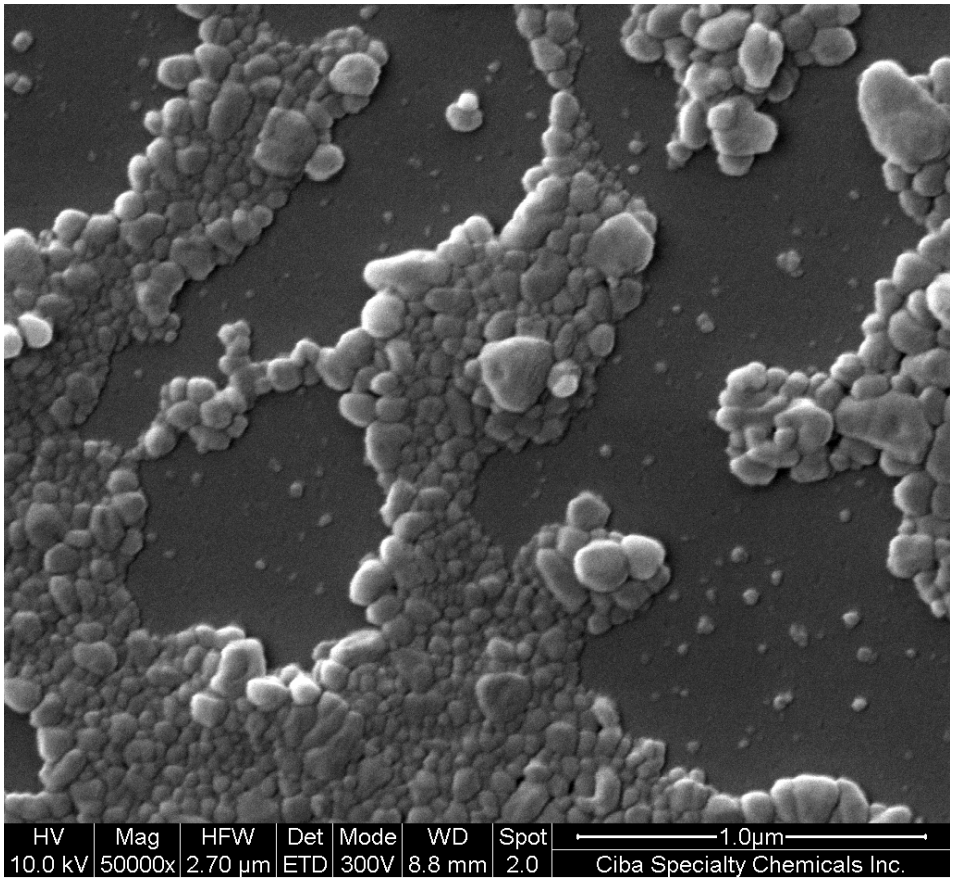


Figure 1: SEM picture of a sample of Tinosorb A2B (active ingredient) OP Lot 07612FC7

Particle Dosing Estimation

Physical characterization parameters of ETH-50 are summarized in Table 4.

Table 4: Particle parameters for ETH50

Parameter	Value*
Assumptions	a. 30% concentration of mono-disperse particles of size d(0.5); b. Values are representative for other ETH50 nano-sized dispersions used in toxicology studies.
d(0.5)	8.7E-08 m
Surface Area	2.38E-14 m²/particle
Volume	3.45E-22 m³
Density	1256 kg/m³
Number particles per cm³	**2.9E+15**
Estimated Weight of one particle	**4.33E-16 g**
Specific Surface Area	**54.9 m²/g**
Values prepared and summarized in appendix to submission III Corrected values are provided in submission VI	

Calculation of particle dosages or exposures calculated with these parameter values are assumed to be representative of the various batches of nano-sized ETH50 used in the toxicology studies reported herein. This is a conservative assumption because only one batch of test item approximated this median particle size; the other batches had a larger median particle size as summarized above in Table 3.

In the following study summaries, the particle doses are derived from the values shown in Table 4.

3.1.5 Solubility

Water solubility (of FAT 65080/B, non-micronized):

< 0.03µg/L at 21°C (OECD 105) insoluble

Ref.: E (subm I)

Other solvents: some examples

Ref.: H (subm I)

Solvent (INCI Name)	Solubility
Cyclomethicone	< 0.001 %
C_{12}-C_{15} Alkyl Benzoate	0.029 %
Caprylic / Capric Triglyceride	0.020 %
Propyleneglycol	< 0.001 %
Mineral Oil	0.003 %
Coco Caprylate / Caprate	0.019 %
Dicaprylyl Carbonate	0.022 %

3.1.6 Partition coefficient (Log P_{ow})

Log P_{ow} (FAT 65080/B): > 5.6 (calculated from the individual solubility in n-octanol and in water)
Log P_{ow} (FAT 65080/B): 10.4 (calculated by a model calculation)

Ref.: E (subm I)

3.1.7 Additional physical and chemical specifications

Appearance: white solid
Melting point: 281.3 °C (OECD 102)
Vapour pressure: $4.15*10^{-21}$ Pa at 25°C, (calculated using the Modified Watson Correlation method; OECD 104)
Flammability: not considered as highly flammable
Explosive properties: Based on its chemical structure, FAT 65'080 is not considered as an explosive material.

Note: the physico-chemical specifications above are provided for ETH50 before the micronizing process.

Ref.: C, D, F, G, (subm I)

In the figure below, it is shown that, as the particle size of the ETH50 decreases there is a corresponding increase in the UV absorption by the smaller particles. C-801 is the marketed product.

UV-Spectrum: Absorption in UVA2 and UVB region increases with the decreasing particle size (Figure 2)

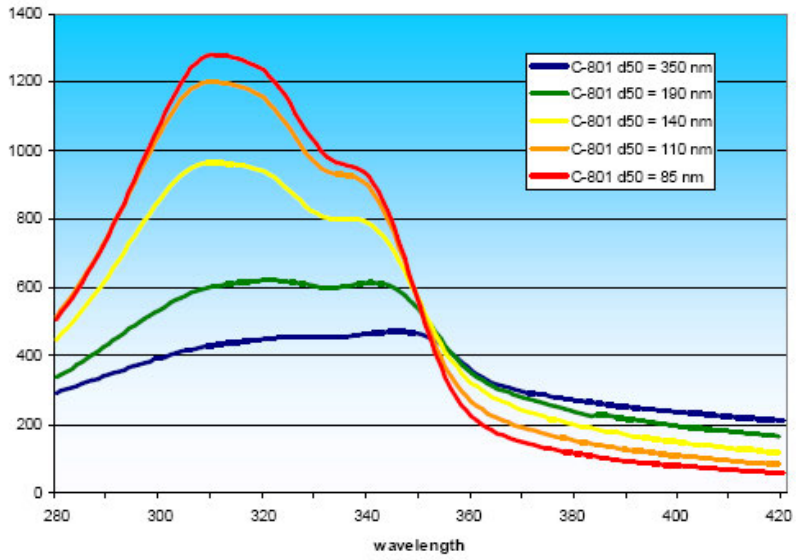


Figure 2: UV-Spectrum

Ref.: submission summary (subm V)

3.1.8 Stability

The substance suspended in 0.5% carboxymethylcellulose is stable and homogeneous in concentrations of 10 and 200 mg/ml over a 9-day storage period at +4 °C and protected from light.

Ref.: 3 (subm I)

The stability of FAT 65'080 in a cream formulation (FAT 65'082/B) used to test for dermal contact irritation, phototoxic, photoallergic and/or contact allergic reactions with normal topical use in human volunteers has been checked by HPLC- DAD with an external standard calibration method.

Ref.: 19 (subm I)

No significant differences of active content of FAT 65'080 were found without irradiation and after irradiation with 3MED of the sunscreen formulation; FAT 65'080 was therefore found to be stable during this study.

Stability of nanosized particles in commercial formulations: A representative commercial formulation, an oil-in-water type formulation, was prepared with a mixture of UV filters and excipients to deliver an SPF 50 sunscreen lotion.

Figure 3 shows the comparative Absorbance spectra for a sample after 8-months storage at room temperature (referred to as manufacture 1) and for a newly prepared formulation (manufacture 2). The shape of each UV absorption spectrum is similar thereby indicating particle agglomeration is not likely to have occurred. The samples showed good stability and representativeness of the UV absorption profile during the commercial preparation process and, for one sample, after 8-months storage at room temperature.

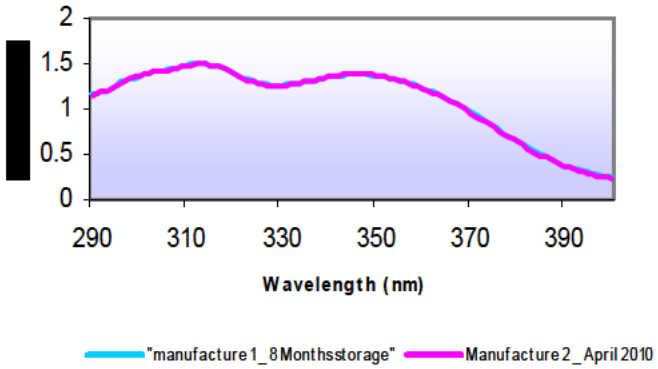


Figure 3: Comparison absorption spectra of sample UV09071-2-7 "manufacture 1" after 8 month storage & "manufacture 2"

Ref.: submission summary (subm V)

3.2 Function and uses

The substance is an active UV filter, intended to be used at concentrations of up to 10% in sunscreen products as skin protectant against UV-A2 and UV-B rays.

3.3 Toxicological Evaluation

The toxicity studies that were initially submitted were carried out with non-micronized material. However, the intended commercial product is micronized to ETH-50 with a d(0.5) of 100-110 nm (in this opinion referred to as nanosized). As the test substance used in toxicological testing should be representative of the marketed material, and moreover a significant fraction of the material is expected to be in the size range for nano-materials (i.e. below 100 nm), studies with nanosized ETH-50 were requested, which were provided in a later submission. In that same submission, additional studies with non-micronized material were provided to directly compare the nanosized and the non-micronized material.

From a safety assessment perspective the studies carried out with the nanosized material are the most relevant, including the studies that were submitted for reasons of comparison between nanosized and non-micronized material. Therefore these studies are described in the main part of the opinion. In Annex I the studies that were performed with the non-micronized and the micron-sized material, are described.

3.3.1 Acute toxicity (nano-sized material)

3.3.1.1 Acute oral toxicity (nano-sized material)

Guideline:	OECD 423 (2001)
Species/strain:	Sprague-Dawley rats
Group size:	Group 1: 3 females; Group 2: 3 females
Test substance:	FAT 65'080/E
Vehicle:	purified water
Batch:	KRG328-2
Concentration:	49.5 % of the active UV filter ETH50
Particle size:	d(0.5) =81 nm
Dose level:	2000 mg/kg bw (both groups) in 0.5% methylcellulose
Route:	Oral, gavage, administration volume 10 ml/kg bw
Observation:	14 days
GLP:	In compliance

Dose in mg/kg bw	Total particles dosed (number)	Surface area of particles dosed (m^2)
2000	**5.8×10^{14}**	**13.7**

Corrected values provided in Submission VI.

Mortality and clinical signs of toxicity were not observed in any of the animals. In 2/6 animals, body weight was slightly reduced. The body weight gain of the other animals was not affected by treatment with the test item. At necropsy, no apparent abnormalities were observed in any animals.
Under the conditions of this study the median lethal dose of the test substance after oral dosing was found to be greater than 2000 mg test substance/kg bw for female rats, corresponding to greater than 1000 mg ETH50/kg bw.

Ref.: 1 (subm III)

3.3.1.2 Acute dermal toxicity (nano-sized material)

No data submitted

3.3.1.3 Acute inhalation toxicity (nano-sized material)

Guideline: OECD 403 (1981)
Species/strain: Wistar [HanRcc:WIST(SPF)] rats
Group size: 15 males/15 females
Test substance: FAT 65'080/FBatch:LOT04122FC7
Concentration: 47.6 % of the active UV filter ETH50 (actual concentration in the study: see below)
Particle size: d(0.5)=109 nm
Duration: 4 hours
Route: Inhalation, nose only
Observation: 14 days
GLP: In compliance

The test item and the placebo were prepared as aqueous dilutions at a ratio (w/w) of 20% FAT 65'080/F or 20% FAT 65'080-placebo plus 80% purified water, the target concentration as active ingredient of UV filter ETH50 was 10%.
Dose level: chemically determined mean aerosol concentration of 4.976 mg of a formulation containing 10% ETH50/L air (s.d. ± 0.513 mg/L air, n = 4), equivalent to a gravimetrically determined mean concentration of 0.602 mg ETH50/L air (s.d. ± 0.059 mg/L air, n = 4). Two gravimetric measurements of particle size distribution during exposure indicated mass median aerodynamic diameters (MMADs) and geometric standard deviations (GSD) of 1.23 µm (GSD 2.13) and 1.27 µm (GSD 2.19).

A group of 15 male and 15 female rats was exposed by nose-only, flow-past inhalation to an aqueous dilution of FAT 65'080/F at a chemically determined mean aerosol concentration of 4.976 mg formulation/L air (s.d. ± 0.513 mg/L air, n = 4), equivalent to a gravimetrically determined mean concentration of 0.602 mg/L air (s.d. ± 0.059 mg/L air, n = 4). Two gravimetric measurements of particle size distribution during exposure indicated mass median aerodynamic diameters (MMADs) of 1.23 µm (GSD 2.13) and 1.27 µm (GSD 2.19) (size of the aerosols). The particle size distribution was determined twice during each exposure using a Mercer 7 stage cascade impactor (Model 02-1300).
An additional group of 15 males and 15 females was exposed to an aqueous dilution of the control item, FAT 65'080 Placebo, at aerosol generation conditions similar to those used for the test item
The first satellite group was sacrificed about 14 hours post end of exposure for broncho-alveolar lavage fluid (BALF) and plasma sampling, the second group was assigned to interim pathology at approximately 24 hours post end of exposure (test day 2), and the third was assigned to pathology at 14 days post exposure (test day 15). All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. until interim sacrifice or over a 15-day observation period. Body weights were recorded before exposure on test day 1 in all animals and during the observation period on test days 4, 8 and 15 in all animals assigned to be sacrificed on day 15.
The BALF examinations comprised total and differential cell counts and the determination of total protein, TNF-α and IL-6. In addition, total protein was determined in blood plasma from the animals assigned to BALF sampling. Pathology examinations comprised complete macroscopic pathology, the determination of lung weight and histopathology of the lungs and tracheobronchial lymph nodes on days 2 and 15.

	MMAD	Median droplet volume cm^3	No Particle a.i./L	Approx. Surface area (m^2) / L
Test item	1.2 µm	9.05E-19	1.15E+12	0.0275
Placebo	1.08 µm	6.60E-19	--	--

Results
Clinical signs attributable to treatment with the aqueous placebo dilution or test item dilution did not occur and no premature deaths occurred during the study.

In BALF collected at about 14 hours after end of exposure the total cell count (mainly macrophage and neutrophil numbers), TNFα and total protein were considerably higher in both sexes of test item-treated animals than in placebo control animals, while total protein levels in plasma did not distinguish the two groups. The results are summarized in Table 5 below. The changes in BALF were consistent with the histopathology findings of granulocytic infiltration in alveolar wall and lumen, diffuse alveolar histiocytosis and alveolar lining cell activation seen in all test item-treated animals assigned to interim pathology at approximately 24 hours post end of exposure (test day 2).

Table 5: BALF Cell Count and Morphology; Cytokines and Protein 24 hours after end of exposure.

Summary (mean ± std dev.)				
Parameter	**Males** (N= 5)		**Females** (N= 5)	
	Placebo	**Test Item**	**Placebo**	**Test Item**
Total Cells (millions)	8.54 (3.03)	62.41**(16.29)	8.67 (0.79)	34.98**(16.68)
% of Total cells				
Macrophages	95.8 (1.4)	26.5 (1.3)[a]	92.5 (3.6)	36.7** (11.8)
Eosinophils	0.2 (0.2)	0.5 (0.7)[a]	0.1 (0.1)	0.3 (0.3)
Lymphocytes	0.8 (0.4)	0.5 (0.7)[a]	0.4 (0.4)	0.4 (0.4)
Neutrophils	0.9 (0.4)	71.0 (2.3)[a]	2.4 (2.4)	60.8**(12.1)
Other cells	0.3 (0.3)	0.9 (1.3)[a]	0.3 (0.2)	0.5 (0.5)
Epithelial cells	2.0 (0.7)	0.6 (0.8)[a]	4.3 (1.4)	1.2 (0.6)
TNFα (pg/ml)	<22.4	61 (17.73)	<22.4	46.2 (7.8)
IL6 (pg/ml)	61[b]	35; 226[c]	283; 35[c]	37; 72[c]
Total Protein (g/l)	68.1 (24.38)	317 (46)	153 (72)	243 (80.6)
Plasma Protein (g/l)	57.45 (2.33)	60 (1.61)	62 (1.2)	62 (2.04)
* / ** t-test based on pooled variance, significant at 5% or 1% level. **a**. N = 2 animals; samples damaged. **b.** 4 rats <24 pg/ml **c.** 3 rats <24 pg/ml				

Increases of total cell count, TNFα and total protein in BALF and of absolute and relative lung weight, and the histopathology findings of granulocytic infiltration, diffuse alveolar histiocytosis and alveolar lining cell activation seen in the test item group on test day 2 were attributed to the treatment with the test item. Increase in neutrophil numbers in BALF on test day 2 was considered to be indicative of an inflammatory reaction.
The study authors considered the findings noted in test item-treated animals on test day 2 to represent an acute clearance reaction to the lung burden of test item. This is further supported by the absence of these findings by test day 15. This response is well documented for particle exposures to lung (Stone et al. 2007)[1] and the cellular response is not characteristic of immunologic response profiles reflected in the low numbers of lymphocytes at day 2 and their absence histologically at day 15 (Holt, P. et al. 2008)[2].
In the discussion of the study results, the applicant argued that such pulmonary inflammatory responses are not expected with use of ETH50 in sunscreens or other products from spray-on dispensers. Spray applicators are generally pump-type dispensers with some increase in marketed products using fine-spray aerosol type dispensers. In each of these applicators, the droplet sizes (aerosol) are designed to be at least 30 times larger than those used in this rat inhalation test. According to Durand et al. (2007)[3], sunscreen sprayable formulations should be dispensed with droplet size of more than 30 µm (MMAD) with not more than 1% of droplets of aerodynamic diameter at or below 10 µm.

The authors concluded that for nanosized ETH50 the inhalation LC_{50} is greater than the highest technically achievable aerosol concentration level of 4.976 mg formulation/L air, or

1 Stone, V. et al. "Proinflammatory effects of particles on macrophages and epithelial cells." Ch 9 in Particle Toxicology, K. Donaldson & P. Borm editors. CRC Press, Boca Raton, FL 2007
2 Holt et al. Regulation of immunological homeostasis in the respiratory tract. Nature Reviews- Immunology. Vol. 8: 142-152, 2008
3 Durand et al. Influence of different parameters on droplet size and size distribution of sprayable sunscreen emulsions with high concentration of UV-filters. Int J Cosmetic Sci. vol 29: 461-471, 2007

greater than 0.4976 mg ETH50/L air, according to this study's results with MMAD of about 1.2 µm. A notable but reversible lung inflammatory response occurred which is considered a normal non-allergenic type response to particle exposures.

Ref.: 5 (subm III)

Comment
The observed effects are similar to those observed upon inhalation of particulate materials in general including some nano-particles. However, the massive influx of neutrophils and the increase of macrophages by approximately a factor 2 cannot be considered to be merely a mild inflammation after inhalation of particles. None of the results in the cited Reference Stone et al. 2007 supports this conclusion. In contrary, these results presented here clearly indicate a strong inflammatory response of the host. The fact that even on day 15 a few parameters differed from those of the controls confirms this strong adverse reaction after inhalation of ETH50 (109).

3.3.2 Irritation and corrosivity (nano-sized material)

3.3.2.1 Skin irritation

Data generated with non-micronized test material only, see Annex I

3.3.2.2 Mucous membrane irritation

Data generated with non-micronized test material only, see Annex I

3.3.3 Skin sensitisation

Data generated with non-micronized test material only, see Annex I

3.3.4 Dermal / percutaneous absorption (nano-sized material)

Additional data available for micron-sized test material ETH50 (440nm), see Annex I

***In vitro* percutaneous penetration study (human and rat skin)**

Guideline	OECD 428
Test substance:	Non-radiolabelled FAT 65'080 + [^{14}C]-labelled FAT 65'080, nanosized with surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol) to mixture of 90.88 mg [^{14}C]-labelled FAT 65'080/ml. Specific activity of the mixture: 1.07 µCi/mg. Concentration in the final formulation: 95.64 mg FAT 65' 080/ml.
Batch:	Non-radiolabelled: KOC00050/004E Radiolabelled: 49336-1-61
Purity:	Non-radiolabelled: 98% Radiolabelled: >99%
Particle size:	d(0.5)= 86 nm (mean of 2 measurements, with values of 92 and 81 nm)
Dose applied:	1.97 mg/cm² in 13 µl for 24 hours
Skin preparation:	Full thickness skin from rats and from humans, removed from subcutaneous fat and upper 200 µm from stratum corneal by dermatome. Pieces of 1.8 x 1.8 cm were mounted in flow-through diffusion cells, with 0.64 cm² of skin membrane exposed to the donor chamber.
Skin temperature:	ambient
Exposure period:	24 hours

Donor chamber: non-occluded
Receptor fluid: 6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v). Solubility: 0.16 g FAT 65'080/ml
Control: No control was used
Skin integrity: 50 µL of tritium water was applied to the skin membrane surface in occluded donor chamber
GLP: in compliance

A target dose level of 2 mg/cm² was selected for both rat and human skin based on the topical application rate of final sunscreen formulations assumed to be used by humans. A 13 µL aliquot of the dosing solution, adjusted to pH 6.5 with 20% citric acid solution, was applied manually to each skin membrane preparation using a µL-syringe. The amount applied to each cell was shown to be 1224 µg/cell or 1912 µg/cm² by determination of the radioactivity content of three control doses taken prior to the first, in the middle, and after the last administration for each dose level. The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions. The receptor fluid was delivered at a flow rate of about 3 mL/h during the testing period. The perfusate from each cell was collected separately at ambient temperature in 1-hour intervals for the 0 to 6 hour period (6 intervals), and in 2-hour intervals for the remaining exposure period (9 intervals).

Twenty-four hours after application the perfusate sampling was terminated and each skin membrane surface rinsed three times with about 0.5 mL shower gel (1%) in water and then with 0.5 ml tetrahydrofuran for each chamber. All skin membrane rinse fractions were combined according to the individual cells. The skin membranes were stripped until the stratum corneum was removed from the skin membrane, which were 6 to 8 strips per membrane. Up to five consecutive stripping tapes were combined into one specimen. The skin membranes remaining after stripping were digested in Solvable and the radioactivity was determined by LSC. The diffusion cells were then washed with 150 mL chloroform and the radioactivity in the cell wash was determined by LSC.

Results
Based on test item found in perfusate the percutaneous penetration rate in rat and human skin was very low or below reliably quantifiable concentrations (LQ) as shown in the following table.

	Rat Skin Membrane		**Human Skin Membrane**	
Applied Dose [µg/cm²]	1971.7		1912	
Applied Volume [µL]	13		13	
Application Area [cm²]	0.64		0.64	
Concentration [mg/cm³]	97		94	
Penetration within	% of dose	µg/cm²	% of dose	µg/cm²
6 h	< 0.01	*0.193	< 0.01	*0.150
12 h	0.02	*0.321	< 0.01	*0.246
24 h	0.02	*0.486	0.02	*0.373
Flux[µg/cm²/h]- measured	0.035		0.026	
Flux[µg/cm²/h]- estimated⁺	0.044		0.042	

* value calculated from the measured dpm, most of which are below LQ of 0.04 µg-equivalents
\+ estimated by replacing <LQ values with the LQ and calculating flux

Considering the solubility of FAT 65080 in the perfusate (0.16 µg/mL), the flow rate of the perfusate (about 3 mL/h), and the exposed skin membrane area (0.64 cm²) the limit for penetration rate due to the solubility of the test item in the perfusate was estimated to be 0.750 µg/cm²/h. This indicated that the perfusate solubility would not be expected to limit

the movement of test item through the skin. There is no information with regard to whether the penetrated substance is present in the form of particles or as solubilised material.

The estimated Flux for rat skin was 0.044 µg/cm²/h, defined as the penetration rate at steady state between 1-6 hours and calculated by using the corresponding LQ values instead of the measured values, most of which were below LQ. Similarly, the Flux for human skin was estimated to be 0.042 µg/cm²/h for 1-6 hours based on the LQ values.

Recovery [% of Dose]*		
Skin Membrane:	Rat	Human
Applied Dose [µg/cm²]	1971	1912
Perfusates Remaining skin membrane	0.02 (0.03) # 1.36 (1.7)	0.02 (< 0.01) # 0.04 (0.07)
Total absorbed (%)	**1.38**	**0.06**
As µg a.i./cm²	**27.2**	**1.2**
Skin membrane rinse	82.73 (8.1)	94.49 (4.3)
Tape strips	13.46 (4.84)	3.73 (2.19)
Diffusion cell wash	0.64 (0.16)	0.66 (0.43)
Recovery	**98.2** (2.29)	**98.94** (3.04)
* values are the mean (± standard deviation) # calculated from measured dpm values, most of which were below LQ of about 0.4 µg a.i. equivalents		

Mean values for absorbed test item of 1.38% and 0.06% of the applied dose were obtained for rat and human skin, respectively.
The very low absorption and percutaneous penetration of the test item allowed only an estimation of a flux parameter because the majority of the values were below limits of quantification for the samples. The rat skin was somewhat more permeable to the test item and showed larger amounts of test item in the tape strips and the remaining skin compared to human skin, whereas from human skin almost 95% of the applied dose could be removed with surface wipe and membrane rinses. Both test systems showed recovery of more than 98% of applied dose and the test item was shown to remain stable during the 24-hour exposure period.

Based on these results, the test item did not penetrate through the skin membranes to a significant extent.
Given the large variability in the absorption values, the mean value ± 2 SD will be used for the calculation of the MOS. This results in a total absorption of $(1.38+ 2x\sqrt{(0.03^2+1.7^2)} = 4.78$ % for the rat and $(0.06+2x\sqrt{(0.01^2 +0.07^2)}= 0.20$% for human skin.

Ref: 2 (subm III)

Comment:
Since most of the measured values were below the LQ, the calculation of the absorption value can be considered conservative.

***In vitro* penetration study with pre-damaged human skin:**

Guideline	OECD 428
Test substance:	Non-radiolabelled FAT 65'080 + [^{14}C]-labelled FAT 65'080, nanosized with surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol). Concentration in final formulation: 95.64 mg [^{14}C]-labelled FAT 65'080/ml. Specific activity of the mixture: 1.07 µCi/mg
Batch:	Non-radiolabelled: KOC00050/004E Radiolabelled: 49336-1-61
Purity:	Non-radiolabelled: 99.1% Radiolabelled: >99%
Particle size:	($d_{0.5}$) = 120 nm

Dose applied: 2.05 mg/cm² in 13 µl for 24 hours
Skin preparation: The subcutaneous fat was carefully removed from the full thickness skin and pieces of about 4 x 5 cm² were stretched evenly over a cork block, with stratum corneum uppermost. Before sectioning three consecutive tape strips were performed upon each of the two skin samples. Skin sections of 200 µm thickness were cut off from the top using a dermatome. Pieces of 1.8 x 1.8 cm were mounted in flow-through diffusion cells, with 0.64 cm² of skin membrane exposed to the donor chamber.
Skin temperature: ambient
Exposure period: 24 hours
Donor chamber: non-occluded
Receptor fluid: 6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v).
Control: none
Particle size: d(0.5)= 120 nm
Skin integrity: 50 µL of tritium water was applied to the skin membrane surface in occluded donor chamber
Recovery: 107.15%
GLP: in compliance

Results

The mean particle size of the test item in this study was ($d_{0.5}$) = 120 nm, which is larger than in the first study with normal skin ((d0.5) = 86 nm). The test item was shown to remain stable during the exposure period. Six of 9 human skin membranes demonstrated acceptable permeability coefficients (mean K_p= 2.69 ± 0.19) and were used for the study.
Based on test item found in perfusate the percutaneous penetration rate in pre-damaged human skin was very low or below reliably quantifiable concentrations as shown in the following table.

	Pre-damaged Human Skin Membrane	
Applied Dose [µg/cm²]	2053	
Applied Volume [µL]	13	
Application Area [cm²]	0.64	
Concentration [mg/cm³]	101	
Penetration within	% of dose	µg/cm²
6h	* 0.75	* 15.315
12h	* 0.75	* 15.483
24h	* 0.76	* 15.658
Flux[µg/cm²/h]- measured*	**0.281**	
* calculated with values below LOQ (~0.05 µg-equivalents)		

Considering the solubility of FAT 65080 in the perfusate (0.16 µg/mL), the flow rate of the perfusate (about 3 mL/h), and the exposed skin membrane area (0.64 cm) the limit for penetration rate due to the solubility of the test item in the perfusate was estimated to be 0.750 µg/cm²/h. This indicated the perfusate solubility would not be expected to limit the movement of test item through the skin.
The estimated Flux was 0.281 µg/cm²/h, defined as the penetration rate at steady state between 1-8 hours and calculated by using the measured values, most of which were below LOQ.

Recovery [% of Dose]*	
Skin membrane:	**Result #**
Applied dose [µg/cm²]	2053
Perfusates	0.76 (1.67)
Remaining skin membrane	0.05 (0.08)
Total absorbed	**0.81**

As µg a.i./cm²	**~15**
Skin membrane rinse	101.14 (16.68)
Tape strips	3.4 (5.21)
Diffusion cell wash	1.79 (3.0)
Recovery	**107.15 (9.27)**
* Values are mean (± standard deviation) # Calculated from measured dpm values, most of which were below LQ of 0.04-0.05 µg a.i. equivalents. Results for Cell 6 are excluded from calculations	

The very low absorption and percutaneous penetration of the test item allowed only an estimation of a flux parameter because the majority of the values were below limits of quantification for the samples. As shown, all (101%) of applied was removable in surface wipe and membrane rinses. Test item recovery was complete and the test item was shown to remain stable during the 24-hour exposure period.
Based on these results, the test item with particle mean diameter of 120 nm did not penetrate through the skin membranes to a significant extent and the damaged stratum corneum did not result in a significantly increased penetration rate of the nanosized test item.

Ref.: 1 (subm IV)

3.3.4.1 Combined repeated dose toxicity study / Reproduction / Developmental Toxicity Screening Test (nano-sized material)

Oral

Guideline: OECD 422
Species/strain: Sprague-Dawley, Crl CD® (SD) IGS BR rats
Group size: 10 males + 10 females per dose (11 weeks old at start of study)
Test substance: FAT 65'080/F
Batch: LOT04122FC7
Concentration: 47.6 %
Particle size: d(0.5) =109 nm
Dose levels: 100, 500 and 1000 mg ETH50/kg bw/day in purified water at 5 ml/kg bw/day
Route: oral, gavage
Exposure period: from before mating until day 4 post partum during lactation period
GLP: In compliance

Test Item Dose as mg a.i./kg bw	**Total particles dosed (number)**	**No. Particles/kg bw**	**Surface area of particles dosed (m^2)**
100	5.8E+13	2.31E+14	1.37
500	2.8E+14	1.15E+15	6.86
1000	5.8E+14	2.31E+15	13.7

Daily dosing, at approximately the same time each day, was by gastric intubation as follows:

For the males:
- for 14 days before mating,
- during the 2 weeks mating and the 2 weeks post-mating periods until sacrifice (maximum of 6 weeks in total).

For the females:
- 14 days before mating,
- during the mating period (maximum of 14 days),

- during pregnancy and lactation, until day 4 *post-partum* inclusive, or until sacrifice for un-mated and non-pregnant females.

Day 1 corresponded to the first day of treatment period.

A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of decyl glucoside, silicon defoamer, xantham gum, and butylene glycol was prepared and labelled FAT 65'080/E-placebo. The placebo was diluted in distilled water and administered at a dose of 420 mg/mL, which was equivalent to that of the group receiving the highest dose of test item.

Clinical signs and mortality were checked daily. Body weight and food consumption were recorded weekly. The animals were paired for mating and the dams were allowed to litter and rear their progeny until day 4 *post-partum*. The total litter sizes and numbers of pups of each sex were recorded after birth, pup's clinical signs were recorded daily and pup body weights were recorded on days 1 and 4 *post-partum*. Parameters for pre- and post-implantation loss, mating, fertility, and gestation were recorded. In parent animals from each group, haematology and blood biochemistry investigations were performed in five male and five female animals at terminal sacrifice. In addition, urinalysis was carried out in five males at terminal sacrifice.

The parent males were sacrificed 2 weeks after the end of the mating period. The body weight and principal organ weights (adrenals, brain, epididymis, heart, kidneys, liver, spleen, testes, and thymus) were recorded, a complete macroscopic *post-mortem* examination was performed and selected organs/tissues were preserved. A microscopic examination was performed on selected organs for five males in control and high-dose groups, with particular attention paid to the male gonads for spermatogenesis staging and morphological structure.
The parent females were sacrificed on day 5 *post-partum* (or on day 25 *post-coitum* for females which did not deliver or 24 days after the end of the pairing period for unmated females) and a complete macroscopic examination was performed. A microscopic examination was performed on selected organs of five delivered females in control and high-dose groups.
The litters were sacrificed on day 5 *post-partum* and were carefully examined for gross external abnormalities and a macroscopic *post-mortem* examination was performed.

Results
No test item- related mortality or clinical signs were observed.
Clinical chemistry, haematology, and urinalysis results showed effects in some parameters of the test-item treated groups when compared to the placebo control group; however, when compared to the vehicle control group, these differences were not statistically or biologically significant and were considered as not attributable to the test item treatment.

Dose-level (mg/kg bw/day)	**0 Purified water**	**0 Placebo (a)**	**100**	**500**	**1000 (b)**
Number of pregnant females	9	7	9	10	9
Number of females surviving delivery	8	7	9	10	9
Mean duration of gestation (days)	21.1	21.1	21.3	21.3	21.2
Mean number of *corpora lutea*	19.0	16.6	19.0	17.0	17.0
Mean number of implantations	16.9	15.7	16.6	16.0	15.6
Mean number of pups delivered	16.1	14.1	15.9	15.3	13.4
Mean number of live pups on day 1 *p.p.*	15.8	14.0	15.4	15.1	13.3

Dose-level (mg/kg bw/day)	0 Purified water	0 Placebo (a)	100	500	1000 (b)
The statistical analysis was performed first between placebo controls and purified water controls and then between placebo controls and dose-levels of 100, 500 and 1000 mg/kg bw/day. (a): one female (N27410) excluded due to absence of evidence of mating. (b): one female (N27440) excluded due to unknown male.					

Reproductive data evaluation showed that neither the mating nor the fertility parameters were adversely affected by the test item treatment or administration of the placebo. However, in the placebo control females, a slightly low mean number of *corpora lutea*, and slightly low mean number of implantations, pups delivered and live pups on day 1 *post-partum* were obtained. The results for all groups are summarized in the table.
The observation of the pups after birth did not reveal any increased incidence of pups dying, adverse effects on the pup body weight gains or influence on the pups' sex ratio; furthermore, gross malformations were not found in any of the pups.
At the *post-mortem* examinations of the F0 generation parent animals, test item treatment-related macroscopic observations were not revealed. None of the differences in organ weights noted between the placebo and control groups or between the test item-treated and the placebo groups were considered to be of toxicological importance.
Qualitative staging for testis did not indicate any abnormalities in the integrity of the various cell types present within the different stages of the spermatogenic cycle. The oestrous stages were not affected by test item or placebo treatments, and microscopic abnormalities were not revealed in the evaluation of the ovarian follicles and *corpora lutea* or in the evaluation of the uterus.
Overall, nanosized ETH50 did not show adverse effects to the systemic toxicity endpoints, mating and reproduction parameters, offspring survival to 5 days of age, or to grossly and microscopically evaluated organs and tissues.
No Observed Adverse Effect Level is 1000 mg ETH50/kg bw/day, the highest dose tested.
Ref.: 6 (subm III), 2 (subm IV)

3.3.4.2 Sub-chronic (90 days) dermal toxicity (nano-sized material)

Dermal

Guideline:	OECD 411
Species/strain:	Wistar Han Crl: WI (GLX/BRL/Han) IGS BRO
Group size:	10 males + 10 females per dose. Five males and 5 females added to untreated and placebo control and both high-dose groups for treatment-free recovery period. Three males and 3 females were added to each test group for toxicokinetics investigations.
Test substance:	FAT 65'080/F
Batch:	LOT04122FC7
Concentration:	47.6% of the active UV filter ETH50
Dose levels:	150, 500 and 1000 mg ETH50/kg bw/day in a mixture of 80% Base ointment, hydrophilic and 20% of 0.5% carboxymethylcellulose (w/v) in purified water
Exposed area:	10% of the clipped body surface area (45-50 cm^2 in males, 30- 35 cm^2 in females according to their age/growth)
Route:	dermal
Exposure period:	13 weeks
GLP:	In compliance
Particle size:	d(0.5)= 109 nm

The animals were housed individually with free access to food and water.
Based on results of a 14-day dermal dose range finding study dosages for the 13-week study were set at the dose-levels as active ingredient of 150, 500 and 1000 mg of ETH50/kg bw/day; the study design is shown in the following table.

Group No.	Treatment		Number of animals	Dose-level[∇] (mg/kg bw/day)	Dose-level[#] (mg/kg bw/day)	Concentration of excipients (mg/mL)	Concentration of Active (mg/mL)
1	Control (untreated)	Principal	15 M 15 F	-	-	-	
2	Control (placebo/vehicle)[(a)]	Principal Satellite	15 M 15 F 3 M 3 F	0	0	440	0
3	Low-dose	Principal Satellite	10 M 10 F 3 M 3 F	150	315	66	60
4	Mid-dose	Principal Satellite	10 M 10 F 3 M 3 F	500	1050	220	200
5	High-dose I	Principal Satellite	15 M 15 F 3 M 3 F	1000	2100	440	400
6	High-dose II (with collar)	Principal Satellite	15 M 15 F 3 M 3 F	1000	2100	440	400

M: male; F: female.
(a): placebo was diluted in the vehicle at the concentration of 840 mg/mL (taking into account the density).
[∇]: expressed as active component FAT 65'080.
[#]: expressed as test item as received (FAT 65'080/F).
-: untreated control group.

For animals of groups 2 to 5, no dressing or protective plastic collar was used. At least 6 hours after each application the dose site was cleaned using purified water and dried with a cotton pad. Animals of group 6 wore a protective plastic collar for a period of at least 6 hours after each application in order to prevent ingestion of the test item. The collar was removed after each exposure period and the application site was cleaned using purified water and dried with a cotton pad. A constant dosage-volume of 2.5 mL/kg bw/day was used.

Mass dosed: mg a.i./kg bw/ day	**% a.i. applied**	**mg a.i. / cm² on dose site**	**Number a.i. Particles/ kg bw/d**	**Number a.i. particles/ cm²**	**surface area (m²) dosed/cm²**
150	6	1.5	3.46E+14	3.46E+12	0.08
500	20	5	1.15E+15	1.15E+13	0.275
1000	40	10	2.31E+15	2.31E+13	0.549
1000	40	10	2.31E+15	2.31E+13	0.549
Estimated Human exposures	10	0.2	6.93E+13	4.62E+11	0.011

Animals of group 1 (untreated control group) received neither treatment nor rinsing but clipping of the application site was conducted as for the other groups. The animals of group 2 (placebo/vehicle control group) received the placebo diluted in the vehicle. The dosage forms were stirred continuously throughout the dosing procedure.

The test item was applied daily for a period of at least 13 weeks (*i.e.* 91 to 92 days according to the necropsy schedule). At the end of the treatment period, the principal animals of each group were humanely sacrificed, except the first five surviving animals of each sex in groups 1, 2, 5 and 6, which were kept for a 2-week treatment-free period. The satellite animals were allocated to toxicokinetics investigations and those animals in groups 5 and 6 were kept for the 2-week treatment-free period.

Results
No effect was seen with regard to mortality, food consumption, ophthalmology, in clinical examination, haematology, blood biochemistry, urinalysis, and in the Functional Observation Battery

Scabs were noted at the application site in about half of the animals in the high dosed group. Clinical signs related to pain, such as abnormal vocalization and/or hyperactivity were observed, mainly from week 5, within the 30-minute period after treatment and generally lasted for less than 30 minutes, in animals given the test item at the high dose-level of 1000 mg/kg bw/day, but mainly in the high-dose group with no protective collar.

In all males given 1000 mg/kg bw/day (groups 5 and 6) a statistically significantly ($p<0.05$) lower mean body weight gain was recorded, principally from week 2 and during the whole study period. This effect was reversed during the treatment-free period. Mean body weight and mean body weight gains for females of both high dose groups (groups 5 and 6) were similar during the full study period.

All group 3 to 6 animals showed quantifiable amounts of the active ingredient of the test item in the plasma during the study period, suggesting a systemic exposure occurred although no time (duration of exposure) or dose-related patterns were demonstrated. The analytic level of quantification was 0.8 ng/ml. Only parent ETH50 was found in the analyzed samples. The results are summarized in the following table.

Plasma ETH50 Concentrations (ng/ml) in 13-Week Dermal Rat Study

	Male					Female				
Group Number	2	3	4	5	6	2	3	4	5	6
Dosage (mg/kg bw/day)	0	150	500	1000 High-dose I	1000 High-dose II	0	150	500	1000 High-dose I	1000 High-dose II
Day 8	0	0.79	1.36	4.17	1.21	0	0.36	5.62	3.04	1.39
Week 13	0	2.39	3.48	2.36	2.47	0	2.05	9.82	2.10	11.81
Week 15 (after reversibility)	*n.a*	*n.a*	*n.a*	1.98	0.39[▽]	*n.a*	*n.a*	*n.a*	0.39	<0.8[#]

[▽]: mean concentration with exclusion of the N26873 value (16.8 ng/mL), considered to be aberrant
n.a: not applicable.
[#] : missing values for animals N27054 and N27055

Organ weight changes were recorded in the thymus (decrease) of group 5 males, and the adrenal glands (increase) of group 5 females. Although no histopathologic changes were observed in these tissues in rats of this dose group, the changes were considered as non-specific indicators of a stress-related response.

Epidermal hyperplasia and associated hyperkeratosis were noted in rats from groups 2, 5 and 6, which received similar doses of excipients either directly or mixed with test item. These findings were considered likely to reflect mild non-specific irritant-effects related to the mechanical preparation of the application site and repeated treatments with equal excipient concentrations to the application site. Minimal increased lymphocytolysis was recorded in a few rats of groups 5 and 6 (*i.e.* mainly females of group 5). This finding may reflect a minimal non-specific stress-related response related to the treatment procedure.

At the dose-levels of 150 and 500 mg/kg bw/day, no signs of local or systemic toxicity were noted.

Under the experimental conditions of this study, the No Observed Adverse Effect Level (NOAEL) of ETH50 (FAT 65'080) is 1000 mg/kg bw/day as active ingredient given by cutaneous application to rats of the FAT 65'080/F test item form during 13 weeks.

Ref.: 7 (subm III), 3 (subm IV)

Comment
The SCCS considers the decrease in body weight in the high dose group as an adverse effect, and therefore sets the NOAEL of ETH50 at 500 mg/kg bw/day.
The animals were suffering pain, indicated by 'Clinical signs related to pain, such as abnormal vocalization and/or hyperactivity'. It is not clear what the reason for this pain was.
It is remarkable that in all dose groups ETH50 was detected in the blood, also in the high dose group animals that wore a collar. Although there is no clear dose-relationship, there is an indication of dermal and/or oral absorption. Although the levels are low and variable, comparison of the plasma levels after day 8 and week 13, and the low levels still present 2 weeks after the end of exposure (week 15), might suggest accumulation of the substance/particles.

3.3.5 Toxicokinetics (nano-sized and non-nanosized material)

In vivo oral absorption, distribution, and elimination- rat

Guideline	OECD 417
Test substance:	Non-radiolabelled FAT 65'080 + [^{14}C]-labelled FAT 65'080, nanosized with surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol) to mixture of 90.88 mg [^{14}C]-labelled FAT 65'080/ml. specific activity of the mixture: 1.07 µCi/mg. 0.8 mL was diluted in 2.4 mL purified water to prepare the oral gavage mixture dosed at 1.0 mL per animal
Batch:	Non-radiolabelled: KOC00050/004E Radiolabelled: 49336-1-61
Purity:	Non-radiolabelled: 98% Radiolabelled: >99%
Dose:	100 mg/kg bw
Species/strain:	Rats, HanRcc:WIST (SPF): Wistar rats
Group size:	four males
Route:	oral, gavage
Particle size:	d(0.5)= 86 nm (mean of 2 measurements)
GLP:	in compliance

This study was conducted immediately after the in vitro percutaneous penetration study and used the same test item and dosing mixtures.

Dose in mg a.i./kg bw	Total particles dosed (number)	No. Particles/kg bw	Surface area of particles dosed (m^2)
113	5.22E+13	2.61E+14	1.24
117	5.40E+13	2.70E+14	1.28

The nominal dose was 100 mg test item per kg body weight.

Urine and faeces samples for each of four 24-hour periods were collected individually and separately per metabolism cage; urine was collected into containers on dry ice, faeces at room temperature and the daily collections stored frozen until analysis. Study termination

at 96-hours after dosing was by CO_2 anaesthesia and exsanguinations, during which blood was collected from each animal.

Cages were rinsed separately. Samples of liver, kidney, renal fat, muscle were taken, and as the remaining carcass, analysed for radioactivity.

Results
All 4 animals survived the study period, gained weight, and did not show signs of toxicity or adverse effects. Administered doses ranged from 113 to 117 mg ETH50 /kg bw with mean of 115.5 mg/kg bw and 912.98 kBq/animal.

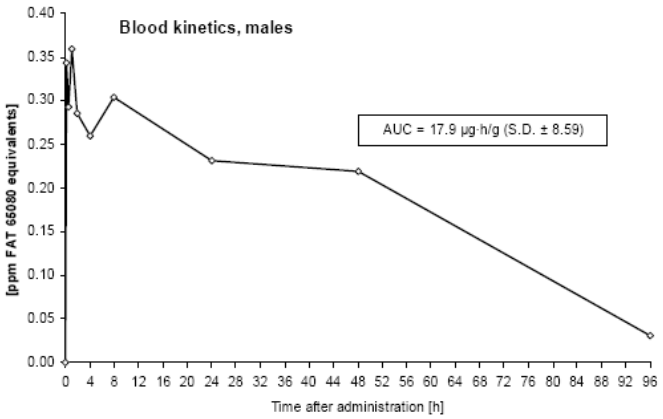


Figure 4: blood kinetics

Absorption into systemic circulation based on urinary excretion was 0.06% of applied dose. The maximum concentration level in blood was achieved 1 hour after administration, accounting for 0.360 µg FAT 65080 equivalents/g. This concentration level remained almost constant until 8 hours post dosing. Thereafter the concentration decreased with a terminal half-life of about 31 hours. The AUC values, being an index of bioavailability, were calculated to be 17.9 µg·h/g for blood. Figure 4 shows the time course for the radioactivity administered with the test item.

Blood and plasma did not show differences in distribution of the test item. Radiolabelled test item did not exceed LOQ in any tissue or organ at 96-hours after dosing; the LOQ was about 0.091 µg-equivalents per gram except for fat, which was 0.182 µg-equivalents/g. Only the remaining carcass (LOQ of 0.012 µg-equivalents/g) showed measurable quantities of test item that approximated 0.07 (± 0.01)% of the applied dose

Excretion		
Urine	Time period	[% of dose]
	0 – 24 h	**0.04**
	24 – 48 h	**0.01**
	48 – 72 h	**<0.01**
	72 – 96 h	**<0.01**
	Subtotal	**0.06**
Faeces		
	0 - 24 h	**92.27**
	24 - 48 h	**1.06**
	48 - 72 h	**<0.01**
	72 - 96 h	**<0.01**
	Subtotal	**93.34**
Cage wash		**0.10**
Total excretion		**93.49**

Elimination was almost fully via faeces and accounted for 93.34% of administered dose or 99.8% of the recovered excreted radioactive material. Urine, representing absorbed radioactive material, was 0.06% of the total amount administered.

Total recovery was 93.56% of administered radioactivity during the study period.

The study authors concluded that there was only minimal absorption for the nano sized (d(0.5) = 86 nm) ETH50 from the gastro-intestinal tract.

Ref.: 3 (subm III)

Comment
Although not specifically required by OECD 417, it would have been informative to analyse other major organs like spleen, lung and brain for radio-activity.
The applicant assumed that the absorption was mainly due to impurities present in the radio-labelled product. Although co-labelling of impurities indeed cannot be excluded, no reason was presented by the applicant why impurities should have a preferred higher absorption than ETH-50 itself. Therefore it is assumed that the measured radioactivity is derived from the absorbed radiolabelled active ingredient.

For comparison between the toxicokinetic behaviour of the particles in nano- and non-nano size, also a ADME study in male rats with particles with a mean size of 6 µm has been carried out:

In vivo oral absorption, distribution, and elimination- rat

Guideline	OECD 417
Test substance:	FAT 65'080 + [^{14}C] labelled FAT 65'080; micronised and filled up with CMC solution (= 0.5% carboxymethylcellulose and 0.4% Tween 80). The final micro suspension had a concentration of 17.4 mg/test item/ml suspension
Particle size:	$d_{0.5}$ =6 µm
Batch:	Non-radiolabelled: KOC00050/004E Radiolabelled: 49336-1-61
Purity:	Non-radiolabelled: 98% Radiolabelled: >99%
Dose:	100 mg/kg bw
Species/strain:	Rats, HanRcc:WIST (SPF): Wistar rats
Group size:	2 groups, group 1 mass balance: 4 rats, group 2 blood kinetics: 9 rats
GLP:	in compliance
Route:	oral, by gavage

[^{14}C] FAT 65080 (d(0.5) = 6 µm) was administered to male rats at a nominal dose level of 100 mg/kg bw. The excretion of radioactivity in urine and faeces was measured in daily intervals up to 96 hours after administration. The concentration of radioactivity in blood, plasma, liver, kidneys, muscle, and fat was determined 96 hours after administration. The blood and plasma kinetics after oral administration was investigated. Additionally the urinary and faecal metabolite pattern was established.
After oral administration the test item was very poorly absorbed from the gastro intestinal tract into system circulation. The extent of absorption, calculated based on the urinary excretion, accounted for 0.73% of the administered dose. Almost the complete dose was excreted unabsorbed with the faeces as unchanged parent compound, accounting for 97.2 % of dose within 48 hours after administration.
The maximum concentration of radioactivity in blood and plasma was achieved 1 hour after administration, accounting for 2.463 and 4.359 µg FAT 65080 equivalents/g, respectively. This plateau level remained constant until 8 hours post dosing. Thereafter the concentrations in blood and plasma decreased with a half-life (8-48 h) of about 13 hours. The AUC values (0-96 h) were calculated to be 65.2 and 114.3 µg·h/g for blood and plasma, respectively.

The tissue residues, 96 hours after administration, were consequently very low. The highest concentration was found in abdominal fat accounting for 1.712 µg FAT 65080 equivalents/g. All other selected tissues and organs revealed concentrations below 0.110 µg FAT 65080 equivalents/g.
The urinary metabolite pattern investigated revealed 7 metabolite fractions. However, the major fraction (U7) represented only 0.19 % of the dose. All other fractions were below 0.1 % of the dose. The faecal metabolite pattern consisted essentially of unchanged FAT 65080.
In summary, after oral administration of [14C] FAT 65080 only a very low amount of radioactivity was absorbed from the gastrointestinal tract and almost the complete dose was excreted unabsorbed as unchanged parent with the faeces.

Ref.: 4 (subm III)

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vivo* (nano-sized and non-nanosized material)

Unscheduled DNA synthesis in hepatocytes, rat

Guideline:	OECD 486
Species/strains:	Fischer rat from Charles River France (males)
Test substance (nanosized):	FAT 65'080/E (49.5% ETH50, batch number KRG328-2, prepared by micronizing ETH50 LOT11106CL4AA in decyl glucoside, xanthan gum, and butylene glycol; subsequent analysis of the test item sample indicated a concentration of 50.6% a.i) particle median diameter d(0.5)= 81 nm.
Test substance (non-micronised):	FAT 65'080/B (ETH50 batch No. KOC00050/004.E, purity: 98%); the median particle size, d(0.5) =15.4 µm.
Test substance (placebo):	a mixture of decyl glucoside, xantham gum, and butylene glycol
Batch:	see above
Vehicle:	water
Expression times:	2-4h and 12-16h after dosing
Concentrations:	nanosized: 0, 500, 1000, 2000 mg/kg bw; 0, 253, 506, 1012 mg a.i./kg bw
Non nanosized:	2000 mg a.i./kg bw
Route:	oral, by gavage
Solubility:	Precipitation of the test substance was found from about 500 µg/plate onward
GLP:	yes

Test Item Dose as mg a.i./kg bw	Total particles dosed (number)	No. Particles/kg bw	Surface area of particles dosed (m^2)
500	2.31E+14	1.15E+15	5.49
1000	4.62E+14	2.31E+15	10.98

Doses were selected based on a preliminary toxicity test to define maximum tolerated doses. Dosing was once by oral gavage at 10 ml/kg bw and each group consisted of 3 males. Positive control substances were dimethylhydrazine at 10 mg/kg bw for the 2-4 hour expression time and 2-acetamidofluorene at 25 mg/kg bw for the 12-16 hour expression time. Blood samples were collected after sacrifice for determination of test item concentrations. Hepatocytes were collected after liver perfusion and removed to culture well-plates for radiolabelling; 12 culture wells per animal were prepared as slides.

Autoradiography was conducted with 6 slides, 6 were held as backup if needed, and where possible 50 cells per slide from 3 slides per animal were evaluated for grain counting classified as nuclear (NC) or cytoplasmic (CC) grain counts, and calculation of net nuclear grains per cell (NNG = NC-CC).

Results

Results of UDS Assay with ETH50 (FAT 65'080)

Assay I: Expression time 12-16 hours

12-16 hours expression time	DOSES in mg/kg	Net Nuclear Grain Count NNG	Net Nuclear Grain Count of cells in repair NNG≥5	% cells in repair NNG≥5	% cells in S-Phase
		Mean	Mean	Mean	Mean
Vehicle control	0	-1.65	5.65	1.49	0.14
FAT 65'080	2000	-1.77	5.63	1.81	0.09
	1000	-1.83	6.19	1.78	0.14
FAT 65'080/E - Placebo	1000	-1.69	5.82	2.17	0.00
FAT 65'080/B	2000	-1.78	5.59	1.55	0.16

Assay II: Expression time 2-4 hours

2-4 hours expression time	DOSES in mg/kg	Net Nuclear Grain Count NNG	Net Nuclear Grain Count of cells in repair NNG≥5	% cells in repair NNG≥5	% cells in S-Phase
		Mean	Mean	Mean	Mean
Vehicle control	0	-1.37	5.53	1.38	0.07
FAT 65'080	2000	-1.09	5.60	1.59	0.08
	1000	-1.28	6.08	1.69	0.09
FAT 65'080/E - Placebo	1000	-1.31	6.53	1.47	0.25
FAT 65'080/B	2000	-0.92	6.12	1.98	0.10

All three tested items (micronised ETH-50, non-micronised ETH-50, placebo) did not cause increased net nuclear grain counts, did not increase the frequency of cells in repair, or induce cellular proliferation as seen in frequency of cells in S-phase. The viability of the hepatocytes was not affected by the in vivo treatment. The positive control items each gave responses indicating a responsive test system.
The genotoxic response from the nano-ETH50 was not different to that of non-nano ETH50. Under the conditions of this study, ETH50, regardless of its particle size, is not mutagenic or genotoxic.

Ref.: 9 (subm III)

Comment
Given the low oral absorption (and dosing by oral gavage), it is not clear whether the hepatocytes have actually been exposed. Therefore, this study is of limited relevance.

3.3.6.2. Mutagenicity / Genotoxicity *in vivo* (nano-sized and non-nano-sized material)

Bone Marrow Micronucleus test

Guideline: OECD 474
Species/strains: Mice, Swiss Ico: OF1 (IOPS Caw) mice
Test substance (nano): FAT 65'080/E. (49.5% ETH50, batch number KRG328-2, prepared by micronizing ETH50 LOT11106CL4AA in decyl

	glucoside, xanthan gum, and butylene glycol; subsequent analysis of the test item sample indicated a concentration of 50.6% ETH50); particle median diameter d(0.5) = 81 nm
Test substance (non-nano):	FAT 65'080/B (ETH50 batch No. KOC00050/004.E, purity 98%); median particle size d(0.5) = 15.4 µm.
Test substance (placebo):	a mixture of decyl glucoside, xantham gum, and butylene glycol
Batch:	see above
Vehicle:	water
Route:	i.p.
Concentrations:	250, 500 and 1000 mg/kg bw/day active ingredient (nano-sized) and 2000 mg/kg bw/day active ingredient (non nanosized)
Solubility:	Precipitation of the test substance was found from about 500 µg/plate onward
GLP:	yes

Test Item Dose as mg a.i./kg bw	Total particles dosed (number)	No. Particles/kg bw	Surface area of particles dosed (m^2)
250	1.73E+14	5.77E+14	0.41
500	3.46E+14	1.15E+15	0.82
1000	6.93E+14	2.31E+15	1.65

A preliminary toxicity test was performed to define the dose-levels to be used for the cytogenetic study. In the main study, one group of five males and five females received the positive control test item (Cyclophosphamide) once by oral route at the dose-level of 50 mg/kg bw. Three groups of five males and five females mice were given intraperitoneal administrations of ETH50 (nano), placebo, or ETH 50 (non-nano) at dose levels cited above. The high dose ETH 50 (nano and non-nano) group retained satellite groups of 3 male and 3 female mice for blood sampling after dosing. Blood samples for these determinations were taken from 3 mice per sex at 1 hour (satellite animals) and 24 hours (at terminal sacrifice on 3 out of 8 animals of each sex) after the second treatment. At the time of sacrifice, all the animals were killed by CO_2 inhalation. The femurs of the animals were prepared and the slides were scored for the number of the micronucleated polychromatic erythrocytes (MPE) in 2000 polychromatic erythrocytes.

Results
No clinical signs and no mortality occurred in the animals of both sexes either given placebo mix (1000 mg/kg bw/day) or test item at 250 and 500 mg/kg bw/day. At 1000 mg/kg bw/day, the ETH 50 (nano) treated group showed hypoactivity and piloerection. The ETH 50 (non-nano) group at 2000 mg/kg bw/day showed hypoactivity, piloerection and soft faeces.
The ETH50 treated males and females had mean values of MPE and a PE/NE ratio that were equivalent to those of the vehicle control group. Also for either placebo or ETH50 (non nano) treated groups, the mean values of MPE were equivalent to those of the vehicle control group. The PE/NE ratio of females treated with the reference item was significantly lower than that of the vehicle control group. Blood samples showed measurable concentrations of test item, both in the nano and in the non-nano groups, indicating that the bone marrow was exposed to the test item.
Cyclophosphamide induced a significant increase in the frequency of MPE, indicating the sensitivity of the test system under the experimental conditions.
In conclusion: the test item, regardless of its particle size did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two i.p. administrations at dose levels of 500-1000 and 2000 mg/kg bw/day. The test item in vivo is not clastogenic or aneuploidic under the conditions of the study.

Ref.: 8 (subm III)

3.3.7 13-Week Phototoxicity study in hairless mice (nano-sized material)

Guideline:	- (The requirements of the U.S. Food and Drug Administration (FDA) were used as the basis for study design)
Species/strains:	Mouse/Crl:SKH1-*hr* (m/f)
Test substance (non-nano):	FAT 65'080/E; d(0.5)= 81 nm
Vehicle:	Aloe Vera Lotion
Route:	dermal
Duration	13 weeks (range finding study)
Concentrations:	0, 25, 50, 100 and 200 mg a.i./g vehicle (0, 80, 160, 325, 650 mg a.i./kg bw/day)
GLP:	in compliance

Eighty four male and eighty four female albino hairless Crl:SKH1-hr mice were randomized to fourteen groups, six mice per sex per group, as outlined in the below study design table.

Group	Description	Formulation Concentration (mg/g)	UVR Exposure per Week (RBU)	Administration Volume (mcl/mouse)
1	No Administration	Not Applicable	None	None
2	Vehicle[a]	0	None	100
3	FAT65'080/E	25	None	100
4	FAT65'080/E	50	None	100
5	FAT65'080/E	100	None	100
6	FAT65'080/E	200	None	100
7	No Administration	Not Applicable	600	None
8	Vehicle[a]	0	600	100
9	No Administration	Not Applicable	1200	None
10	Vehicle[a]	0	1200	100
11	FAT65'080/E	25	1200	100
12	FAT65'080/E	50	1200	100
13	FAT65'080/E	100	1200	100
14	FAT65'080/E	200	1200	100

[a] Aloe Vera Lotion
Abbreviations : UVR : Ultraviolet Radiation ; RBU : Robertson-Berger Units

The ultraviolet radiation (UVR) source used was a 6.5 kilowatt xenon long arc, water cooled burner vertically-suspended within an octagonal metal frame holding one optical filter on each side. Each filter (15 cm by 15 cm, 1 mm thick; Schott WG 320 doped glass) was held approximately 20 cm from the burner. The racks holding the mouse cages were located approximately 2.25 meters from the UVR source during exposure. Each rack of cages was irradiated through one filter; all racks of cages are irradiated simultaneously from one xenon arc. Each rack of animal cages was monitored by a customized detector that records both intensity and UVR dosage in Robertson-Berger Units (RBU). The RBU is a measure of skin response to UVR; 400 RBU approximates one minimal erythema dose (MED) on previously untanned human skin.
The test item was ETH50 (LOT11106CL4AA) nanosized in decyl glucoside, xanthan gum, and butylenes glycol and labelled as FAT 65'080/E (KRG328-2); after this study was completed the test item sample was again evaluated for purity (50.6% active ingredient) and particle size distribution characterized as d(0.5)=81 nm; d(0.9)=157 nm. Dosages are expressed as active ingredient (a.i.) and summarized in the following table.

Mg a.i./g dose formulation	% a.i.	mg a.i. / cm² on dose site	Approx. mg a.i./kg bw/ day	Approx. No. A.i. particles/ cm²	Specific surface area (m²)/cm²
25	2.5	0.1	80	2.3E11	0.005
50	5.0	0.2	160	4.6E11	0.010
100	10	0.4	325	9.2E11	0.020
200	20	0.8	650	18.4E11	0.040
Approximate Human Exposures	10	0.2	60	4.6E11	0.011

The test article formulations and/or vehicle were administered at a dosage volume of 100 ul/mouse and the appropriate mice were irradiated once daily, 5 days per week, for 13 weeks as outlined in the Study Design Tables. Formulations were administered to the back and sides (approximately 25 cm²) of appropriate mice before daily UVR exposure on Monday, Wednesday and Friday and after UVR exposure on Tuesday and Thursday. On Monday, Wednesday and Friday, UVR exposure began no later than 15 minutes after the completion of formulation administration for each group. On Tuesday and Thursday, the duration of time between the completion of UVR exposure and the start of formulation administration for each group of mice was no longer than 15 minutes.

Clinical observations and skin observations were recorded at least once weekly. Erythema, oedema, flaking, or any other abnormal findings were recorded when observed and the intensity of these observations is described in terms of an internationally accepted standard, which is a modified Draize system. Body weights were recorded at least weekly throughout the study. Skinfold thickness for each mouse was measured using a thickness gauge before administration of any formulation (0 week) and in weeks 4, 8 and 13.

After completion of the 13-week dosage period, the mice were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Gross lesions found were retained in neutral buffered 10% formalin for possible histopathological examination. Samples of skin from the site of formulation administration for each mouse (or the equivalent anatomical location for untreated mice) were processed for possible histopathological examination. These histopathological examinations were not performed; the remainder of the carcass was discarded without evaluation.

Results
Test article residue was observed in all groups of male mice, without or with UVR exposure administered the FAT65'080/E formulations, as compared with the group of mice not administered any formulation. This finding is a characteristic of the test article and not considered adverse.

All male and female mice survived to scheduled sacrifice. The only necropsy finding in male mice was a tan mass in one mouse not administered any formulation and exposed to 200 RBU/week of UVR and one male mouse administered 50 mg/g FAT 65'080/E and exposed to 1200 RBU/week of UVR. These findings were considered not related to the UVR exposure or FAT 65'080/E administration. All other tissues appeared normal.

Over the course of the study, no significant differences in group mean body weight occurred across the groups of male or female mice administered the vehicle formulation or the FAT 65'080/E formulations, without or with UVR exposure, as compared with groups not administered any formulation or administered vehicle formulation, without or with UVR exposure. Instances of significant changes (increases and reductions) in group mean body weight occurred, as compared with groups not administered any formulation or administered vehicle formulation, without or with UVR exposure. These changes in group mean body weights were not considered related to formulation administration and/or UVR exposure or biologically important because: 1) the occurrences were not administration

volume-dependent; 2), the changes were both increases and reductions; and/or 3) the occurrences were intermittent.

For male and female mice throughout the study, there were scattered instances of significant changes in mean skinfold thickness changes in both male and female mice administered the formulations, without or with UVR exposure, as compared with groups not administered any formulation without or with UVR exposure. These significant changes were not considered adverse because of the lack of dose and time-dependence and lack of a pattern of response that would indicate a clear association of the test item with this clinical endpoint.

The topical administration of nanosized ETH50 with and without UV radiation did not adversely affect oedema formation, wrinkling, or skin fold thickness in any group of males or females; these endpoints showed an increase in male and female mice treated with the vehicle alone or with UVR alone. This indicates ETH50 in nanosized formulation does not generate alternative toxic forms or increase the adverse effects of UV irradiation and leads to a decreased incidence of markers of UV radiation damage indicative of an efficacious protective effect.

Ref.: 10 (subm III)

3.3.8 Human data (nano-sized material)

Human phototoxicity and photoallergenicity test

Guideline:	/
Subjects:	57 subjects, of which 53 completed the study: 35 women and 18 men, age 19 - 56 years, mean age 35.1 years (SD = 10.9)
Test substance:	1. CD05-123-01 (Cream formulation containing 9.9% FAT 65'082/B) 2. CD05-123-02 (Placebo cream) 3. CD05-123-03 (FAT 65'082/B Placebo)
Batch:	Unknown
Concentration:	9.9%
Particle size:	d(0.5)= 90 nm
UV radiation:	UVA+UVB from a 150 watt xenon arc solar simulator (Solar Light Company, Philadelphia, PA), equipped with a Schott WG320 UVC blocking filter, a heat-rejecting dichroic mirror and a visible and infrared blocking UG-11 filter. A UVB blocking filter was used for UVA doses. The lamp beam was uniform, as evidenced by uniform erythema across exposed sites, with a continuous spectrum that was free from substantial peaks. Less than 0.01% of total lamp energy was contributed by wavelengths shorter than 290 nm. The irradiation doses were 10 Joules/cm² of UVA followed by 0.5 MEDs of UVA/UVB radiation.
Dose levels:	20 mg in Finn chambers (0.5 cm²) on midback secured with tape. Subsequent application of 2 µl/cm² directly on skin.
Route:	topical
Observation period:	Immediately, 24±4, 48±4 and 72±4 hours after each application / irradiation
GCP:	In compliance

The design of the study was an open-label, controlled study, consisting of six duplicate, occluded exposures to test products in Finn Chambers, followed by UVR administration to half the sites, over a two-week Induction Phase; a 9-14 day Resting Phase and a single 24 hour application of the test products in a Challenge Phase. In the Challenge Phase, test products were applied to sites not previously treated, half the sites were irradiated with UVR and responses were evaluated 48 and 72 hours after removal of patches.

Thirteen subjects reported 16 adverse experiences, none of which were considered related to the test substance as that were similar to both controls. Based on these results, the study authors considered that the test substance showed no significant potential for phototoxicity or photoallergenicity.

Ref.: 19 (subm I)

3.3.9 Special investigations

Exposure Studies

The purpose of this investigation was to evaluate the influence of different formulation types and technological parameters such as spray type (propellant or pump spray) or propellant gas concentration on the size and size distribution of droplets in a sunscreen emulsion containing nanosized ETH50 as the only UV filter in the formulation.
Two different types of fluid o/w cosmetic emulsions were prepared containing nanosized ETH50 (mean diameter 124 nm) at a high concentration of 8 and 10%, respectively (formulation names: GEUV10079-1-2 and GEUV10079-2-2). In addition, a third formulation was prepared as a variation of the second. Only Magnesium aluminium silicate was omitted in this emulsion (GEUV10079-2-3).

These formulations were incorporated in aerosol cans with gas (a blend of propane and butane). The propellant was added at a final concentration of 30 and 40%, respectively. The size and the particle size distribution of the droplets were analysed using a Malvern Mastersizer (Malvern, UK). The first and the second formulation were also incorporated into cosmetic pump spray bottles. The type of aerosol valves and spray heads were of typical quality and specification for cosmetic aerosols, e.g. hair sprays (supplier: Precision Company, USA). The particle size was characterized by the mass median diameter d(0.5). The measuring distance of droplets was set at 30 cm, because it was considered as a relevant distance under normal use conditions.

Results
The results of the spraying experiments with the ETH50 formulations are summarized in the table below.

Influence of the formulation type:
Formulation 1 produced larger droplets than formulation 2 and 3 (e.g. the d(0.1) values were about five times higher compared to formulation 2 and 3 for the propellant sprays).

Influence of the propellant concentration:
As it could be expected, 40% propane/butane lead to finer aerosols compared to a concentration of 30% propellant.

Influence of the spray type (pump spray versus propellant spray):
The pump spray containing formulation 1 produced smaller droplets than the propellant spray with the same formulation. As expected, the pump spray containing formulation 2 generated larger droplets compared to the propellant spray.
For both pump sprays, the droplet fraction below 10 µm was below 1%. For the propellant spray with formulation 2 and 3 this fraction was well below 10%.

Table 6: Droplet sizes for different propellant and pump sprays using laser diffraction (single measurements for pump sprays and propellant sprays 40%, duplicates for propellant sprays 30%)

Formulation	o/w Emulsion (GEUV10079-1-2)			o/w Emulsion GEUV10079-2-2			o/w Emulsion GEUV10079-2-3
Conc. Butane/Propane	30%	40%	0% Pump-spray	30%	40%	0% Pump spray	40%
Conc. ETH50 in formulation	8%	8%	8%	10%	10%	10%	10%
Mean Diameter (D0.5, volume distribution)	210/229 µm	204 µm	132 µm	82 / 78 µm	57 µm	68 µm	50/52 µm
Mean Diameter (D0.1, volume distribution)	110/128 µm	100 µm	53 µm	29 / 29 µm	21 µm	40 µm	16/19 µm
Max. fraction of droplets below 10 µm	<1%	<1%	<1%	< 10%	< 10%	<1%	< 10%

Conclusion:
The mean droplet size (d(0.5); volume distribution) for all spray formulations (propellant sprays and pump sprays) using laser diffraction methodology (Malvern Mastersizer) was found to be about 50 µm or above. The droplet fraction below 10 µm was found to be well below 10% (propellant sprays) and 1% (pump sprays), respectively.

Ref.: 2 (subm VI)

The release of aerosols from 3 pressurised ETH50 spray formulations was investigated with a Scanning Mobility Particle Sizer (SMPS) regarding their particle size distribution in the sub-micron range range between 10 and 600 nm. The atomisation process was operated in an enclosed box to get constant conditions and a particle free background. An SMPS equipped with a sample holder for TEM-grids was used in order to sample particle fractions and perform an element analysis by a Transmission Electron Microscope (TEM) and EDX (Energy Dispersive X-Ray)-spectroscopy. A SMPS for online recording of particle distributions was used, and a second CPC to monitor the particle concentration in the glove box during the whole measurement time. A closed environment with minimal particle background is needed to perform reliable spray experiments. The closed glove box setup ensured stable conditions and avoided external influences such as air flow. The setup assured a minimal particle background environment with maximum of 10 particles per cm^3 in the measured size range between 10 nm and 600 nm. The particle evacuation after a spray experiment required around 5 minutes of flushing the glove box with nitrogen until reaching acceptable background levels. Before each experiment, the spray cans were shaken 20-times by hand and a 2-minute measurement period with the SMPS followed. The recording time was divided into 1 minute of measurement with spraying followed by 1 minute of measurement without spraying.

The investigated formulations were the same as for the Mastersizer experiments. However, only the 40% propane/butane sprays were tested in the SMPS experiments as they were considered a worst case scenario based on the laser diffraction data.

The first formulation (GEUV10079-1-2) showed particle number concentrations lower than 1000 particles/cm³.
The two other formulations showed particle concentrations above 1·106 particle/cm³. These differences might be explained by the different composition of the three formulations. The third sample (GEUV 10079-2-3) was investigated after the second formulation (GEUV 10079-2-2) with the aim to elucidate the increased particle number concentration seen with the second formulation. The signals detected might have been caused by tiny particles of Magnesium aluminium silicate, which was contained as an ingredient in this formulation. Therefore, the third formulation was prepared without Magnesium aluminium silicate. However, the submicron fraction was still observed with this formulation.

In order to identify the chemical nature of the submicron particles or droplets detected with formulation 2 and 3, two particle size fractions of about 120 nm and 370 nm were then collected on a TEM grid sampler that was connected to the SMPS. The TEM-images and EDX-spectra were taken from the third formulation only, as the composition of the second and the third formulation was very similar except the presence of Magnesium aluminium silicate in the second formulation (GEUV 10079-2-2).

The TEM-grids were prepared in deep frozen sample holders (-180 °C) to avoid a sublimation or vaporisation of low volatile components under high vacuum. The results showed that no organic particles were present on the TEM-grid. ETH50 was not found in the EDX-spectra. Magnesium aluminium silicate was also not identified. The submicron droplets or particles detected by the sensitive SMPS have therefore to be of volatile nature whereas ETH50 has a high molecular weight (MW 538) and a very low volatility (calculated: 4.15x10-21 Pa at 25 °C).
One explanation for the observation of volatile droplets or particles in the submicron range may provide the findings of Chen et al. (1995) who reported that nanoparticles in a range of around 10 nm can be generated through water nucleation in a saturated atmosphere by number concentrations of cN≥1051/cm³ is therefore considered possible. It could be speculated that a coagulation of low volatile nanoparticles (liquid or solid) in the range between 10 nm to 600 nm might have occurred with the o/w sunscreen emulsions, which was then detected by the sensitive SMPS system.

In conclusion: The SMPS results showed that the presence of submicron ETH50 particles or agglomerates could not be detected in any of the spraying experiments. By EDX and TEM analysis, it could be demonstrated that the submicron fraction, which was observed with one of the formulation types, consisted of volatile molecules or particles. They were most likely formed by water/oil nucleation processes during spraying or either could originate from impurities from the cosmetic raw materials used.

Ref.: 3 (subm VI)

Exposure calculations (inhalation exposure):

The applicant has provided an exposure assessment using the ConsExpo model. The current models of ConsExpo has not yet been validated for the use of nanomaterials in spray products. However, the applicant has performed a number of exposure studies in which they have determined relevant exposure parameters for a pump spray and a propellant spray containing nano-sized ETH50.

In addition, an exposure calculation was performed in which it was assumed that particles dry after spraying (thus increasing the number of inhalable particles).

ConsExpo estimations for pump and propellant sun sprays containing 10% ETH50 in a sunscreen formulation (see ref 1, 4 and 5 (subm VI))

Exposure	Pump Spray	Propellant Spray	Propellant Spray (worst case, dried particles)
Mean event concentration (5 minutes)	0.32 mg/m^3	1.26 mg/m^3	5.47 mg/m^3
Acute internal dose by inhalation	0.000607 mg/kg bw	0.00238 mg/kg bw	0.0104 mg/kg bw

The cut-off level for inhalation was set at 15 µm. In the worst case scenario (dried particles) the inhalable fraction (fraction particles <15 µm) was larger compared to the 'normal' situation, therefore the mean event concentration is accordingly higher.

See Annex 2 for the ConsExpo calculations

3.3.10 Safety evaluation (including calculation of the MoS)

Dermal exposure

ETH50 has low oral bioavailability From the in vivo ADME study with ETH50, particle size d(0.5) = 86 nm, it was shown that absorption after oral exposure was < 1% of the administered dose. Therefore, for a risk assessment based on route-to-route extrapolation, the NOAEL from the oral 13 week rat study has to be re-calculated to an internal dose, which would lead to a MOS value below 100.

The SCCS is of the opinion that in the case of substances with very low bioavailability, route-to-route extrapolation is not an appropriate approach and prefers to use the dermal 90 day study for the calculation of the Margin of Safety.

Comparative MoS calculations for ETH50 based on human skin in vitro study results for nanosized (80 nm) and micronized particle size (440 nm) on normal skin

Parameter	ETH50 d(0.5) = 80nm	ETH50 d(0.5) = 440nm
Adult Body weight	60 kg	60 kg
Body surface area	17.500 cm²	17.500 cm²
Sunscreen applied (if at 1 mg/cm²)	18 g	18 g
ETH50 applied (10%)	1800 mg	1800 mg
Skin absorption (human) RCC B236 24 April '07 RCC A00112 August '05	0.20% of applied dose	0.57% of applied dose
Systemic Exposure Dose (human)	0.06 mg/kg bw/day	0.171 mg/kg bw/day

NOAEL Rat 13-wk dermal study (CIT 32404 TCR, Aug' 08)	500 mg/kg bw/day	Not available; applicant used 1000 mg/kg bw/day for study performed with d(0.5)= 15 µm
Skin absorption (rat) (RCC B23624 April '07)	4.28% of applied dose	12.77% of applied dose
Systemic Exposure Dose (rat)	21.4 mg/kg bw/day	127.7 mg/kg bw/day
SED Rat / SED Human	**357**	**746**

Based on the comparison of the internal dose between rat and man, the MoS is 357 for ETH50 with a d(0.5)= 80 nm.
It should be noted that the above calculations are very conservative, in particular with regard to the skin absorption value used. Most values in the dermal absorption assay were below the limit of quantification, but used for the calculation of the penetration. Moreover, the majority of the dose was recovered from the skin compartment, rather than the receptor fluid.

Exposure via inhalation

The applicant has provided a calculation of the margin of exposure. The basis for this calculation is an exposure assessment using ConsExpo. The current models of ConsExpo has not yet been validated for the use of nanomaterials in spray products. However, the applicant has performed a number of exposure studies in which relevant exposure parameters for a pump spray and a propellant spray-can have been determined.

Calculation of Margin of Exposure by comparing the lung burdens in rat and human

Parameter	**Pump spray**	**Propellant spray**	**Propellant Spray (dried particles)**
Mean event concentration (5 min)	0.32 mg/m^3	1.26mg/m^3	5.47 mg/m^3
Human ventilation rate	33 m^3/d= 1.375 m^3/h	33 m^3/d= 1.375 m^3/h	33 m^3/d= 1.375 m^3/h
Exposure time consumer	5 min	5 min	5 min
Inhaled alveolar amount	0.32 mg/m^3 x 5 min/60 min x 1.375 m^3/h = 0.0367 mg	1.26 mg/m^3 x 5 min/60 min x 1.375 m^3/h = 0.144 mg	5.47 mg/m^3 x 5 min/60 min x 1.375 m^3/h = 0.627 mg
Human lung weight	ca. 1 kg	ca. 1 kg	ca. 1 kg
Human lung burden (per g lung)	0.0367 mg/kg lung = 0.0367 µg/g	0.144 mg/kg lung = 0.144 µg/g	0.627 mg/kg lung = 0.627 µg/g
Rat lung burden (per g lung) (see also chapter 1 A)	1.17 mg/g	1.17 mg/g	1.17 mg/g
Margin of Exposure (lung burden rat / lung burden human)	1.17 mg/g/ 0.0367 µg/g **= 31 880**	1.17 mg/g/ 0.144 µg/g **= 8 125**	1.17 mg/g/ 0.627 µg/g **= 1866**

The magnitude of the MoE was calculated in the presented user scenarios by comparing the modelled air concentrations or the internal doses or the lung burdens after the potential inhalation of ETH50. In particular, a comparison of the lung burden gave a MoE of 31880 for pump sprays and 8125 for propellant sprays. If a complete evaporation of the volatile components with a subsequent shrinking of the droplets is assumed, a MoE of 1866 can be calculated for the propellant spray. This MoE is calculated using an effect level (serious lung effects after acute inhalation for 4 hours). It should be noted that a conservative exposure scenario was used (5 minutes exposure, sprayed towards a person (scenario of a hairspray), sprayed in a small room, low air ventilation) and ETH50 was not identified in two measured aerosol fractions.

3.3.11 Discussion

This dossier presents toxicology studies for ETH50. ETH50 is intended to be used in commercial preparations in a nanosized form with a mean particle size around 100 nm.

The studies in the first submission, except for the dermal absorption studies, were carried out with a suspension of non-nanosized ETH50. The dermal absorption studies were carried out with so-called 'micronized' ETH50 having a mean particle size of about 440 nm. Upon specific enquiry, it was confirmed that the mean particle size of 'non-micronised' ETH50 was about 15 µm, but in the formulation to which the consumer is to be exposed the mean particle size would be about 80-100 nm.
Subsequently, new studies were submitted, which were carried out with nanosized ETH50 (d(0.5) of around 80-110 nm), equivalent to the commercialised material. These studies comprised an acute oral and inhalation study, a screening repeated dose/ repro/ developmental toxicity study, a dermal penetration study, an oral ADME study, a 13-week dermal toxicity study and genotoxicity studies. In the toxicological assessment, the results of studies with non-nano material will be compared to the studies carried out with the nano-materials.
It should be noted that the risk assessment for ETH50 is based on mass- dose metrics, and not on particle number or surface area. At this moment the most adequate dose metric for hazard and exposure characterisation for nanoparticles is still under discussion. However, as cosmetic ingredients are usually assessed and regulated based on the concentration in the finished product, this dose metric is maintained in this opinion.

Acute toxicity
In an acute inhalation study with ETH50 (d(0.5) = 109 nm) no lethality was observed. However, a strong inflammatory response was seen in the lung of exposed animals.
The acute oral toxicity of 49.5% nano-ETH50 d(0.5) = 81 nm was >2000 mg/kg bw (corresponding to >1000 mg ETH50/kg bw).

The oral and the dermal toxicity of non-nanosized ETH50 (d(0.5) = <15.4 µm) was >2000 mg/kg bw.

Irritation /sensitisation
ETH50 (d(0.5) = <15.4 µm) does not show irritating properties to the eyes or to the skin. It is not sensitising in an LLNA test. No studies were performed with nanosized ETH50.
Given the very limited systemic exposure, the fact that there are no indications for irritation from the dermal 90 day study carried out in rats with nanosized ETH50, and there were no signs of irritation and photoallergenicity in the human study it is not considered necessary to request new irritation/sensitization studies with nanosized ETH50.

Dermal absorption
In an *in vitro* dermal penetration study with nanosized particles (d(0.5)= 86nm), the mean absorption for rat skin was 1.38% or 27.2 µg/cm², and for human skin these values were 0.06% or 1.2 ug/cm². Given the large variability seen in these studies, the mean + 2 SD

were used for the calculation of the MoS. This results in a total absorption of 4.78% for the rat and 0.20% for human skin (it should be realized that these are very conservative values, given the low dose (levels around the limit of quantitation) that could be recovered in the perfusate).
An additional study was performed, in which ETH50 (d(0.5)= 120 nm) was applied to pre-damaged human skin. Again, the concentrations that could be measured were very low (below the limit of quantitation). The mean *in vitro* dermal absorption under these conditions was 0.81% or 15 µg ETH50/cm².
Although under conditions of damaged skin, an increase of dermal absorption can be noted, given the very low levels this is not interpreted as a concern.

The penetration of nanosized ETH50 seemed about a factor 10 higher through damaged skin compared to the penetration through intact skin. However, the results of both tests are rather uncertain, since the majority of the values used for the calculation were below the limit of quantification. For the conventional assessment of UV filters dermal absorption is tested on intact skin only. Given the conservative estimate of the dermal absorption, the uncertainty in the penetration values, the severe damage to the human skin in the in vitro experiment and the fact that in the exposure assessment it is assumed that the total body surface area is exposed, the results of the damaged skin are not taken into account for the calculation of the MoS.

In an *in vitro* study using rat skin and human skin (see annex), dermal absorption of non-nanosized ETH50 (d(0.5)= 440 nm) was higher that the absorption with the nanosized ETH50. Using the mean absorption + 2 SD results in a dermal absorption for the rat of 12.77% and for human skin of 0.57%. It should be noted that these are a very conservative values.

Repeated dose toxicity
In a study in which rats were dermally exposed to ETH50 (d(0.5)= 109 nm) for 13 weeks no signs of local or systemic toxicity were noted at dose-levels of 150 and 500 mg ETH50/kg bw/day. In the highest dose group (1000 mg ETH50/kg bw) a decrease in body weight gain was noted. In view of that, the SCCS considers 500 mg ETH50/kg bw/day to be the NOAEL in this study.
In a combined oral repeated dose/repro-/developmental toxicity screening study, no adverse systemic effects were observed after exposure to 1000 mg ETH50/kg bw/day (d(0.5)=109 nm).
In a 90 day oral gavage study (see annex) in rats that were exposed to non-nano sized ETH50, d(0.5) = <15.4 µm, the NOAEL (No Observed Adverse Effect Level) for systemic repeated dose toxicity under the experimental conditions of the study was 1000 mg/kg bw/day.

Mutagenicity/Genotoxicity
An UDS test in rat hepatocytes and an in vivo mouse bone marrow assay, using ETH50, d(0.5)= <15.4 µm, and ETH50, d (0.5)= 81 nm, did not show genotoxicity. However, given the low oral absorption (and dosing via gavage), is was not clear whether in the UDS test the cells were adequately exposed.
An Ames test, an in vitro mammalian chromosome aberration assay and a mouse TK locus in vitro mutation assay, carried out with non-nanosized ETH50 d(0.5)=<15.4 µm did not show genotoxicity.
ETH50 (d(0.5)= 15.4 µm) did not result in photo-genotoxic effects in bacteria and in V79 Chinese Hamster cells.
Overall, the data indicate no concern with regard to potential genotoxicity of ETH50.

Carcinogenicity
No study available

Reproductive and developmental toxicity
In a combined repeated dose/repro-/developmental toxicity screening study, no adverse systemic effects were observed after exposure to 1000 mg ETH50/kg bw/day d(0.5)=109 nm.
In a developmental toxicity study in rats, non-nanosized ETH50 (d (0.5)= 15µm) did not result in maternal or developmental toxicity up to 1000 mg/kg bw/day.

Toxicokinetics and metabolism
Two *in vivo* ADE studies were carried out in rats dosed with ETH50, one with a particle size of d(0.5)=87 nm, the other with d(0.5)=6 µm. The study with the 6 µm radiolabelled particles showed a higher absorption percentage of radioactivity, and higher levels of radioactivity in various tissues. However, the observed absorption was low. In the study using nanosized particles, about 0.06% of the dose was recovered in urine, and only the remaining carcass showed ETH50 above the LOQ (levels in carcass were about 0.07% of the applied dose). From the results presented, accumulation cannot completely be excluded, since half life time seems to be longer than 24 h. Also in the 13 week dermal study, an increase in blood levels is observed over time. It is not clear in which organs the particles accumulate. Unfortunately not all organs have been analysed for residues. For the larger particles (d(0.5)=6 um) 0.73% of the dose was recovered in the urine. Radioactivity was recovered in a number of organs at low levels, however only levels in fat (0.01% of the dose) and the remaining carcass (0.25% of the dose) could be reliably quantified.
In a dermal *in vivo* study (particle size d(0.5)=440 nm, see Annex) absorption was estimated to be about 0.15% of the applied dose.

Phototoxicity
In a dermal 13 week toxicity study, hairless mice were exposed to dose levels of 80, 160, 325 and 650 mg ETH50/kg bw/day (d(0.5)=81 nm) and were daily exposed to UV light. This dose was about 4 times higher than anticipated human topical dose rates from sunscreen application. No increase in oedema formation, wrinkling, or skin fold thickness was observed, compared to control animals.

In a human study, 57 subjects were exposed (six cycles of application and irradiation) to a cream formulation containing 10% ETH50 (d(0.5)=100 nm) and were irradiated with UV light. Phototoxicity or photosensitization responses did not occur.

Inhalation
Inhalation exposure to ETH50 was calculated using ConsExpo. However, this model has not been validated yet for applications with nanosized particulate material. Moreover, only an acute inhalation study (one dose level) was available. From this study and the exposure calculations it could be concluded that there is a relative large margin of exposure between the lung load of the rat and humans. However, no information is available on the effects of ETH50 after repeated exposure to lower doses via inhalation. From other inhalation studies using nano-materials, it is known that the nano-material is not cleared from the lung in the same way as is the case for 'conventional' substances. In addition, the effect of the formulation on the behaviour of the ETH50 particles in the human lung is not known.
Therefore, at the moment, it cannot be concluded that the use of 10% ETH50 in spray formulations is safe, because of the uncertainties associated with the repeated exposure of the lung to low doses of ETH50.

In view of the noted effects in the lung, to ensure the safe use in spray application the following information is needed: a 28-day repeated dose inhalation study, including tissue distribution and systemic toxicity, determination of an LOAEL and NOAEL and possible recovery.

Accumulation of ETH50 in the 13-week dermal study could not be excluded. Given the very low levels (around the limit of quantification), the exaggerated exposure scenario and the

relative large difference in internal dose between man and rat, this is not considered as a concern for ETH50.
However, it is recommended that detailed studies be carried out to characterise the physico-chemical form of the substance (solubilised on in nano form) in tissues and blood, and to investigate possible accumulation in tissues and organs.

The suitability of the currently available toxicological testing methods for nano-materials is still under discussion. For some of the standard methods employed for ETH50, the relevance of the results might be debatable (e.g. *in vivo* UDS test with oral administration, where exposure of the target tissue has not been demonstrated). In addition, the test materials in the assays (*in vitro* and *in vivo*) have not been characterised in the various vehicles and body fluids. Therefore it is not clear to which material (nanoparticles or aggregates/agglomerates or solubilized material) the cells and/or the animals were exposed. However, despite the possible shortcomings of the methodologies, and in view of the very low levels of absorption of ETH50 through dermal route, the SCCS is of the opinion that the submitted data provides sufficient basis for assessment of the safety of ETH50.

It is, however, of note that the potential ecotoxicological impacts of ETH50 when released into the environment have not been considered in this opinion.

4. CONCLUSION

1. *Does the SCCS consider the use of 1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl- in a concentration up to 10% w/w in sunscreen products safe for the consumer?*

The SCCS is of the opinion that:

Dermal exposure to formulations containing the UV-filter ETH50 with a mean particle size of 81 nm, as described in the dossier, results in low absorption of ETH50. Also after oral exposure, absorption of ETH50 is low. No systemic effects are observed after oral or dermal exposure up to 500 mg/kg bw/day. Due to the low bioavailability of ETH50, a risk assessment based on a NOAEL from oral studies and applying route-to-route extrapolation was not considered appropriate. Based on comparison of the internal dose in man and rat (resulting in a MoE of 357) and comparison of the NOAEL in the 13 week dermal study in the rat and the human systemic exposure dose it was concluded that the use of 10% ETH50 can be considered safe for dermal application.

The risk assessment of nanomaterials is evolving. It should be noted that the testing of the substance and the present assessment are based on methodologies initially developed for toxicity testing of substances in non-nano form and current knowledge. From this perspective it is concluded that the use of 10% ETH50 can be considered safe for dermal application. This assessment, however, is not intended to provide a blue-print for future assessments, where depending on the developments in methodology and risk assessment approaches and probable development of nano-specific testing requirements, additional/different data could be required and/or requested on a case-by-case basis.

2. *Does the SCCS foresee any other restrictions to the safe use of 1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-?*

At this moment there is too much uncertainty to conclude about safe use of 10% ETH50 in spray applications, because of concerns over possible inhalation exposure.
Therefore, the SCCS concludes that spray products containing ETH-50 cannot be recommended until additional information on safety after repeated inhalation is provided.

5. MINORITY OPINION

REFERENCES

Submission I, 2005

A. Ciba Specialty Chemicals D-79630 Grenzach-Wyhlen. Test number AO5-1152 (16.09.05). Report on analytical certification with FAT 65080/A.
B. Ciba Specialty Chemicals D-79630 Grenzach-Wyhlen. Test number AO5-1152 (16.09.05). Report on analytical certification with FAT 65080/B.
C. RCC Ltd, CH-4452 Itingen. Study Number 859219 (July, 22 ,2005). Determination of the melting point/melting range and the boiling point/boiling range of FAT 65080/B.
D. RCC Ltd, CH-4452 Itingen. Study Number 859221 (July 19, 2005). Calculation of the vapour pressure
E. RCC Ltd, CH-4452 Itingen. Study Number 859223 /September 26, 2005). Determination of the water solubility and the partition coefficient (n-octanol/water) of FTA 65080/B
F. RCC Ltd, CH-4452 Itingen. Study Number 859224 (August 11, 2005). Determination of the flammability of Fat 65080/B
G. RCC Ltd, CH-4452 Itingen. Study Number 859225 (July 19, 2005). Expert statement on the explosive properties of FAT 65080/B.
H. Ciba Specialty Chemicals Inc., CH-4002 BASEL. List of solubilities of the active ingredient of C-801 (20 July 2004).
1. CIT F-Evreux, Laboratory Study number 26394 TAR (21 November 2003). Acute oral toxicity study in rats "Acute Toxic Class Method" with FAT 65'080/A
2. CIT F-Evreux, Laboratory Study number 26395 TAR (21 November 2003). Acute dermal toxicity study in rats with Fat 65'080/A
3. CIT F-Evreux, Laboratory Study number 28341 TCR (07 September 2005). 13-week toxicity study by oral route (gavage) in rats followed by a 4-week treatment-free period with FAT 65'080
4. CIT F-Evreux, Laboratory Study number 26396 TAL (12 November 2003). Primary dermal irritation study in rabbits with FAT 65'080/A
5. CIT F-Evreux, Laboratory Study number 26397 TAL (02 December 2003). Primary eye irritation study in rabbits with FAT 65'080/A
6. CIT F-Evreux, Laboratory Study number 26399 TSS (16 January 2004). Evaluation of Skin Sensitization Potential in Mice using the Local Lymph Node Assay (LLNA) with FAT 65'080/A.
7. CIT F-Evreux, Laboratory Study number 26400 TSG (22 January 2004). Phototoxic and photoallergenic Potential by Cutaneous route in guinea pigs of FAT 65'080/A
8. Central Toxicology Laboratory, Alderley Park Macclesfield, UK- Cheshire. CTL/XR7473/Regulatory Report (15 June 2005). FAT 65'080/B: In vitro androgen receptor binding assay
9. Central Toxicology Laboratory, Alderley Park Macclesfield, UK- Cheshire. CTL/XR7474/Regulatory Report (15 June 2005). FAT 65'080/B: In vitro estrogen receptor binding assay
10. Central Toxicology Laboratory, Alderley Park Macclesfield, UK- Cheshire. CTL/RR1054/Regulatory Report (10 June 2005). FAT 65'080/B: Uterotrophic assay in immature female rats
11. CIT F-Evreux, Laboratory Study number 28343 RSR (06 September 2005). Study for effects on embryo-fetal development by oral route (gavage) in rats with FAT 65'080

12. RCC Ltd, CH-4452 Itingen, Study number A00112 (03 August 2005). [14C] FAT 65080 (ETH50) Percutaneous Penetration of Micronized [14C] FAT 65080 (ETH50) Through Rat and Human Split-thickness Skin Membranes (in-vitro)
13. (ETH50) Dermal Absorption of Micronized [14C] FAT 65080 (ETH50) in the Rat (in vivo)
14. CIT F-Evreux, Laboratory Study number 26390 MMT (30 December 2003). Bacterial reverse mutation test with FAT 65'080/A.
15. CIT F-Evreux, Laboratory Study number 28643 MLH (05 August 2005). In vitro mammalian chromosomal aberration test in cultured human lymphocytes with FAT 65'080/B
16. CIT F-Evreux, Laboratory Study number 28644 MLY (18 August 2005). In vitro mammalian cell gene mutation test in L5178 TK+/- mouse lymphoma cells
17. RCC Ltd, CH-4452 Itingen, Study number 868701 (April 01, 2005). Photomutagenicity in a Salmonella typhimurium reverse mutation assay with FAT 65'080/B
18. RCC Ltd, CH-4452 Itingen, Study number 868702 (July 04, 2005). Chromosome Aberration test in vitro: Photomutagenicity in Chinese hamster V79 cells with FAT 65'080/B
19. Suncare Research Laboratories, LLC, Winston Salem, NC 27106USA. Study number: SRL2005-089 (October 7, 2005. Human Phototoxicity and Photoallergenicity test withn FAT 65'082/B.

Submission II, 2006

No references attached

Submission III, 2008

A. Ciba Grenzach GmbH, Analytics, D-79630 Grenzach-Wyhlen. Analytical Certificate with FAT 65080/D. Dr. Edith Wieser (23.07.08)
B. Ciba Grenzach GmbH, Analytics, D-79630 Grenzach-Wyhlen. Analytical Certificate with FAT 65080/F. Dr. Edith Wieser (23.07.08)
C. Ciba AG, Trace Analysis & Issue Analytics, CH-4002 BASEL. Quantification of ETH-50 2nd Peak – Order Number 08.183. Denis Hundsbuchler (31 July 2008)
D. Ciba Grenzach GmbH, D-79630 Grenzach-Wyhlen. Identity and Characterization of the Sample FAT 65080/E. Dr.Bernd Herzog (13.02.2007).
E. Ciba Grenzach GmbH, D-79630 Grenzach-Wyhlen. Identity and Characterization of the Sample FAT 65080/F. Dr.Bernd Herzog (28.02.2007).
F. RCC Ltd Environmental Chemistry & Pharmanalytics CH-4452 Itingen/Switzerland Determination of the particle size distribution of FAT 65080/B. Study number 859228; 09 September 2005
1. CIT 2007-a. Acute oral toxicity in rats, acute toxic class method. CIT study no. 32808 TAR; 11 September 2007.
2. RCC 2007-a. [14C] FAT 65080 (ETH50) Percutaneous Penetration of [14C] FAT 65080 (ETH50), Micronized According to Technical Specifications, Through Rat and Human Split-thickness Skin Membranes (in-vitro). RCC study no. B23624; 1 November 2007.
3. RCC 2007-b. [14C] FAT 65080 (ETH 50): Absorption, Distribution and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. B23613; 1 November 2007.
4. RCC 2007-c. [14C] FAT 65080 (ETH 50): Absorption, Distribution, Metabolism and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. A89280; 4 July 2007.
5. RCC 2007-d. 4-Hour Acute Inhalation Study in Rats with FAT 65'080/F. RCC study no. B38698. 16 November 2007
6. CIT 2008-a. Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. CIT study no. 32799 RSR. 30 July 2008.

7. CIT 2008-b. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008.
8. CIT 2007-c. Bone Marrow Micronucleus Test by Intraperitoneal Route in Mice. CIT study no. 32753 MAS. 16 June 2008.
9. CIT 2007-d. In vivo/in vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with FAT 65'080. Institute Pasteur de Lille report no. FSR-IPL 070101. 16 June 2008.
10. CRL-2007-e. Thirteen-Week Topical Range-Finding Study of FAT65'080/E in Hairless Mice, With or Without Simulated Sunlight. Charles River Laboratories Preclinical Services, Horsham, PA, USA; study number XAW 00001; 13 June 2007.
11. Wieser, E. 2008. Report on Analytical Test no.: 08/0662. Ciba Chemicals, PA Research & Technology Analytics. 29 July 2008.

Submission IV, 2009

1. RCC 2008. [14C] FAT 65080 (ETH50): Percutaneous Penetration of [14C] FAT 65080 (ETH50), Micronized According to Technical Specifications, Through Pre-damaged Human Split-thickness Skin Membranes (in-vitro). RCC study number C08835; 29 October 2008.
2. CIT 2008-a. Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. CIT study no. 32799 RSR. 30 July 2008. Final report amendment number 1 dated 4 December 2008.
3. CIT 2008-b. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008. Final report amendment number 1 dated 14 November 2008.
4. CIT 2007-c. Bone Marrow Micronucleus Test by Intraperitoneal Route in Mice. CIT study no. 32753 MAS. 16 June 2008. Final report amendment number 1 dated 13 March 2009.
5. CIT 2007-d. In vivo/in vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with FAT 65'080. Institute Pasteur de Lille report no. FSR-IPL 070101. 16 June 2008. Final report amendment number 1 dated 14 January 2009.

Submission V, 2010

1. Mueller, S., B. Herzog, J. Giesinger, K. Quass, U. Osterwalder. Micronization as a Tool in the Development of Innovative UV Filters. SOFW-Journal 131 7-2005.
2. Herzog, B., Giesinger, J. 2007. Calculation sheet for Specific Surface Area of C-801 Particles (Active substance ETH50). 12 October 2007. Included in text of this submission.
3. Herzog, B. 2010. Determination of the specific surface area of Tinosorb® A2B. BASF internal report dated 6 April 2010.
4. Herzog, B. 2010. Determination of the zeta-potential of Tinosorb® A2B. BASF internal report. 12 April 2010.
5. BASF Tinosorb A2B commercial fomulation UV09071-2-7
6. Herzog, B. 2010. Determination of the extinction of Tinosorb A2B in presence and absence of carboxymethylcellulose (CMC). BASF internal report 12 April 2010.
7. Durand et al. Influence of different parameters on droplet size and size distribution of sprayable sunscreen emulsions with high concentration of UV-filters. Int J Cosmetic Sci. vol 29: 461-471, 2007
8. RCC [14C] FAT 65080 (ETH 50): Absorption, Distribution and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. B23613; 1 November 2007.

9. RCC 2007-c. [14C] FAT 65080 (ETH 50): Absorption, Distribution, Metabolism and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. A89280; 4 July 2007.
10. RCC 2005. Dermal Absorption of Micronized [14C] FAT 65080 (ETH50) in the Rat (in vivo). RCC study no. A22432.
11. CIT 2008. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008.
12. Rat plasma concentration for individual animals in 13-Week dermal dosing study; CIT 32040 TCR.

Submission VI, 2011

1. BASF SE, 2010a.Calculation of lung burden of ETH50 in the 4-hour acute inhalation toxicity study in rats with FAT 65'080 (RCC report, B38698) using Multiple-Path Particle Dosimetry Model (MPPD v 2.1)
2. BASF SE, 2010b.Droplet sizing of sun sprays containing a new cosmetic UV filter (ETH50) using a Laser Diffraction system (Malvern Mastersizer)
3. BASF SE, 2010c. Measurement of the droplet size distribution of sun screen formulations containing ETH50, report date 12 Dec 2010
4. BASF SE, 2010d. ConsExpo 4.1 report, ETH50 Pump Spray (10% ETH50), report date 19 Dec 2010
5. BASF SE, 2010e. ConsExpo 4.1 report, ETH50 Propellant Spray (10% ETH50), report date 18 Dec 2010
6. Bellmann et al, 1992. Irreversible pulmonary changes induced in rat lung by dust overload. Env. Health Perspectives, 97, 189-191
7. Bremmer et al, 2006. Cosmetic Fact Sheet To assess the risks for the consumer, updated version for ConsExpo 4, RIVMreport 320104001, page 18 pp, available from the following internet site: http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf
8. Chen et al, 1995. Electro spraying of conducting liquids for monodisperse aerosol generation in the 4 nm to 1.8 µm diameter range. J Aerosol Sci., 26, 963-977
9. CIT, 2008a. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008
10. CIT, 2008b. Rat plasma concentration for individual animals in 13-week dermal dosing study; CIT 32404 TCR, page 44 and pages 997-1011, 1 August 2008
11. CIT, 2008c. Bone marrow micronucleus test by intraperitoneal route in mice. CIT 32753 MAS. 16 June 2008
12. ConsExpo 4.1. Consumer Exposure and Uptake Models. Available from the following internet site: www.rivm.nl/en/healthanddisease/productsafety/Main.jsp
13. ECHA, 2008 Guidance on information requirements and chemical safety assessment. ChapterR.11: PBT Assessment, May 2008. available online http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r11_en.pdf?vers=20_08_08
14. Hagendorfer et al., 2010. Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. J Nanopart Res 12, 2481–2494
15. Landsiedel, 2008. Short term inhalation tests with 8 nanomaterials in rats. Presentation at the annual SOTmeeting (March 2008). Available from the following internet site: http://www.basf.com/group/corporate/en/sustainability/dialogue/in-dialogue-withpolitics/nanotechnology/knowledge/safety-research
16. Landsiedel et al., 2010. TestingMetal-Oxide Nanomaterials for Human Safety. Adv. Mater., 22, 2601-2627
17. RCC, 2005a. Determination of the water solubility and the partition coefficient (n-octanol / water) of FAT 65080/B. RCC study no. 859223, 26 Sept. 2005
18. RCC, 2005b. [14C] FAT 65080 (ETH50). Percutaneous Penetration of Micronized [14C] FAT 65080 (ETH50) Through Rat and Human Split-thickness Skin Membranes (in-vitro). RCC study no. A00112, 03 August 2005
19. RCC, 2005c. Dermal Absorption of Micronized [14C] FAT 65080 (ETH50) in the Rat (in vivo). RCC study no. A22432

20. RCC, 2007a. FAT 65'080/F4-Hour Acute Inhalation Toxicity Study in Rats, RCC study no. B38698, 16 Nov 2007
21. RCC, 2007b. [14C] FAT 65080 (ETH50) Percutaneous Penetration of [14C] FAT 65080 (ETH50) Micronized According to Technical Specifications Through Rat and Human Split-thickness Skin Membranes (in-vitro). RCC study no. B23624, 01 Nov 2007
22. RCC, 2007c. [14C] FAT 65080 (ETH 50): Absorption, Distribution and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. B23613; 1 Nov 2007.
23. RCC, 2007d. [14C] FAT 65080 (ETH 50): Absorption, Distribution, Metabolism and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. A89280; 4 July 2007.
24. Sayes et al., 2007. Assessing toxicity of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary toxicity profiles. Toxicological Sciences, 97, 163-180

Annex 1: Studies performed with ETH50, mean particle size 15 µm, unless stated otherwise.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 423 (1996)
Species/strain:	Sprague-Dawley rats
Group size:	Group 1: 3 males; Group 2: 3 males + 3 females (approx. 8 weeks old at start of study)
Test substance:	FAT 65'080/A
Batch:	ETH 50/129B
Purity:	> 98%
Dose level:	200 and 2000 mg/kg bw (group 1 and 2, respectively), in 0.5% methylcellulose
Route:	Oral, gavage, administration volume 10 ml/kg bw
Observation:	14 days
GLP:	In compliance

At 200 mg/kg bw, no clinical signs were observed. At 2000 mg/kg bw, piloerection and dyspnea, together with hypoactivity in females, were observed in all animals on day 1.
When compared to historical control animals, a slight reduced body weight gain was recorded in 2/3 males given 200 mg/kg bw during the first or second week of the study. At 2000 mg/kg bw, a reduced body weight gain was noted in 1/3 females during the second week of the study. The overall body weight gain of the other animals was not affected by the treatment with the test substance.

Under the conditions of this study the median lethal dose of the test substance after oral dosing was found to be greater than 2000 mg/kg bw for male and female rats.

Ref.: 1 (subm I)

3.3.1.2. Acute dermal toxicity

Guideline:	OECD 402 (1987)
Species/strain:	Sprague-Dawley rats
Group size:	5 males + 5 females (approx. 8 weeks old at start of study)
Test substance:	FAT 65'080/A
Batch:	ETH50/129B
Purity:	> 98%
Dose level:	A single dose of 2000 mg/kg bw placed on a hydrophilic gauze pad, applied to an area of the skin representing approximately 10% of the total body surface, under semi-occlusive dressing and restraining bandage
Route:	Topical
Exposure period:	24 hours
Observation:	14 days
GLP:	In compliance

A white coloration of the skin was noted in all animals on day 2. No other cutaneous reactions were recorded during the study.

Under the experimental conditions of this study, the median lethal dose of the test substance after topical dosing is higher than 2000 mg/kg bw in rats. No signs of toxicity were observed at this dose.

Ref.: 2 (subm I)

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404 (1992)
Species/strain:	New Zealand White Rabbits
Group size:	3 males (2-4 months of age)
Test substance:	FAT 65'080/A
Batch:	ETH50/129B
Purity:	98%
Dose level:	Single topical application of 0.5 g to intact skin under semi-occlusion for 4 hours
Route:	Topical
Exposure period:	4 hours
Observation:	72 hours
GLP:	yes

In the first instance, the test item was applied for periods of 3 minutes and 4 hours to a single male New Zealand White rabbit. Since the test item was not severely irritant on this first animal, it was then applied for 4 hours to two other animals.
A single dose of 500 mg of the test item in its original form was applied to the closely-clipped skin of one flank.
The test item was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.
The mean values of the scores for erythema and oedema were calculated for each animal.

Results
After a 3-minute exposure (one animal), a very slight erythema was observed on days 1 and 2. After a 4-hour exposure (three animals), except for a very slight erythema noted in 1/3 animals one hour after the removal of the dressing, no cutaneous reactions were recorded during the study.
Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

Conclusion
Under the experimental conditions, the test item FAT 65'080/A is non-irritant when applied topically to rabbits.

Ref.: 4 (subm I)

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand White albino rabbits
Group size:	3 males
Test substance:	FAT 65'080
Batch:	ETH50/129B
Purity:	> 98%

Dose level:	Single application of 0.1 g of the test article as a powder in the conjunctival sac of the left eye, lower and upper eyelids were held together for about 1 second. 24 hours after application, eye was rinsed
Route:	Ocular
Exposure period:	24 hours
Observation:	72 hours
GLP:	In compliance

The test substance was administered as a powder by placing 0.1 g of the powder into the conjunctival sac of the left eye and the lower and upper eyelids were held together for about 1 second. The right eye served as the untreated control. The eyes of the three animals remained unrinsed for approximately 24 hours after instillation of the test article. Indication of pain did not occur in any animal upon instillation of the test article or shortly thereafter. Residual test article was not noted in the treated eyes of any of the animals at the 1-hour observation interval.

A very slight or slight chemosis (grade 1 or 2), a very slight or slight redness of the conjunctiva (grade 1 or 2) and/or a clear discharge, were observed in all animals from day 1 up to day 2 or 3. A slight iritis was also noted in 1/3 animals on day 1. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.3, 0.0 and 0.3 for chemosis, 1.0, 0.3 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and 0.0 for iris lesions and 0.0, 0.0 and 0.0 for corneal opacity.
The substance was slightly irritant to the eye under the conditions of the test

Ref.: 5 (subm I)

3.3.3. Skin sensitisation

Local Lymph Node Assay in mice

Guideline:	OECD 429 (2002)
Species/strain:	CBJ/A mouse
Group size:	4 females (age ±8 weeks)
Test substance:	FAT 65'080/A
Batch:	ETH50/129B
Purity:	>98%
Dose level:	Positive control: 25% α-hexacinnamaldehyde
Vehicle:	propylene glycol (due to unsatisfactory solubility of the test item in acetone/olive oil and in dimethylformamide) Test substance: 0.5, 1.0, 2.5, 5.0 or 10%
Application:	Quantity of test formulation applied: 25 µl
Treatment scheme:	Test item was applied to the dorsum of both ears daily for 3 consecutive days followed by 2 days without treatment. On days 1, 2 and 3 as well as on day 6 (after sacrifice), thickness of the left ear of each animal of the vehicle and treated groups was measured using a micrometer. Any irritation reaction (erythema and oedema) was recorded in parallel.
Preparation procedure:	Termination: On day 6, the mice were injected intravenously with 3H-TdR, and five hours later the mice were sacrificed, and the auricular lymph nodes were excised.
Isolation lymph nodes:	Excised and pooled for each experimental group by mechanical disaggregation in Petri dishes with the plunger of a syringe. Cell suspensions were washed with 15 mL of 0.9% NaCl, pellets re-suspended in 0.9% NaCl for numeration of lymphocytes and determination of viability. Centrifuged and precipitated with 5% TCA (+4 °C) overnight. Centrifuged and precipitated with 5% TCA, addition of 3 mL Ultima GoldxR scintillation fluid (Packard).
GLP:	In compliance

This study was based on the design adopted by ICCVAM (Interagency Coordination Committee on the Validation of Alternative Methods, ICCVAM 1999) and ECETOC (Technical Report No. 78 Skin sensitization Testing: Methodological Considerations, Brussels, December 1999), with the addition of the evaluation of local irritation.

A homogeneous dosage form preparation was obtained at the maximum concentration of 10%. No cutaneous reactions and no increase in ear thickness were observed in the animals of the treated groups. A positive lymphoproliferative response (SI >3) was noted at the concentration of 0.5%. However, as no positive response and no dose-response relationship were observed at the highest tested concentrations, this positive response could not be considered as biologically relevant. It is concluded that under these experimental conditions, the test item FAT 65'080/A does not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.

Ref.: 6 (subm I)

3.3.4. Dermal / percutaneous absorption

In vitro Percutaneous absorption – rat skin, human cadaver skin ex vivo

Guideline:	OECD 428 (2004)
Test substance:	Non-radiolabelled FAT 65'080 + [^{14}C]-labelled FAT 65'080, specific activity 2.11 µCi/mg, micronized with surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol) to mixture of 90.88 mg [^{14}C]-labelled FAT 65'080/ml
Particle size:	d(0.5) = 440 nm
Batch:	Non-radiolabelled: KOC00050/004E Radiolabelled: 49336-1-61
Purity:	Non-radiolabelled: 98% Radiolabelled: >99%
Dose applied:	2 mg/cm² in 13 µl for 24 hours
Skin preparation:	Full thickness skin from rats and from humans, removed from subcutaneous fat and upper 200 µm from stratum corneal by dermatome. Pieces of 1.8 x 1.8 cm were mounted in flow-through diffusion cells, with 0.64 cm² of skin membrane exposed to the donor chamber.
Skin temperature:	/
Exposure period:	24 hours
Donor chamber:	non-occluded
Receptor fluid:	6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v). Solubility: 0.16 g FAT 65'080/ml
Control:	No control was used
Skin integrity:	50 µL of tritium water was applied to the skin membrane surface in occluded donor chamber
Recovery:	95.48 ± 1.49 (rat skin) 91.83 ± 5.38 (human skin)
GLP:	in compliance

The skin membranes were set up in flow-through diffusion cells, the formulated [^{14}C] FAT 65080 was applied onto the skin membranes at a finite dose of 13 µL/cm² and the perfusates collected at defined time intervals.
The formulated [^{14}C] FAT 65080 was applied onto skin membranes of 200 µm thickness at a concentration of 94 mg/cm³ leading to an area concentration of 1912 µg/cm² (reflecting a concentration of 10 % test item in the final formulation).
For each species 7 replicates were used, one cell with human skin was excluded because of a kp > 2.5X10^{-3} cm/hr. The exposure of the test item was performed under non-occluded conditions over an exposure time of 24 hours. During the exposure period the receptor fluid

(6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) dissolved in physiological saline (0.9% NaCl w/v)) and was collected in hourly intervals between 0-6 hours and thereafter in 2 hours intervals until the end of the experiments. At the end of the experiment the remaining [^{14}C] FAT 65080 was removed from the skin membranes by rinsing the skin membranes three times with a mild soap solution. The skin membranes were removed from the diffusion cell and consecutively stripped until the stratum corneum was removed from the skin membrane.

During 24 hours of exposure only 0.12% of the applied dose penetrated through rat skin membranes into perfusate. At the end of exposure the bulk of the applied test item could be washed off from the skin membranes, accounting for 70.57% of the dose. After skin membrane rinsing 20.92% of the dose remained in/on the skin membrane. The major part of this remaining test item was located in the stratum corneum (tape strips), accounting for 15.97% of the dose, and 4.95% of the dose was found in the remaining skin membrane after tape stripping, indicating that the applied test item entered the skin membrane but it did not penetrate through the membrane to a significant extent. The mean flux value was calculated to be 0.209 µg/cm²/h. The flux value was about 3 times lower than the estimated penetration rate limit caused by the very limited solubility of the test item in the perfusate, i.e. 0.750 µg/cm²/h.

For the human skin membrane the penetration of the test item resembles very closely to that observed in rat skin membrane. Within 24 hours of exposure only 0.10% of the applied dose penetrated totally through human skin membranes into perfusate. Also for human skin membranes the bulk of applied test item could be washed off 24 hours after start of exposure, i.e. 73.18% of dose. In stratum corneum 15.38% of the dose was found and 0.18% of the dose was found in the remaining skin membrane after tape stripping, which was significantly lower than observed in rat skin membranes. The mean flux value was calculated to be 0.178 µg/cm²/h.
The amount of test item in lower skin layers was significantly lower in human skin membranes as compared to rat skin membranes. Therefore the total absorption, based on the amount penetrated through the skin membrane (perfusate) and the amount measured in the remaining skin membrane layers after tape stripping, was 5.07% and 0.28% of the applied dose, or 96.9 µg-eq/cm² and 5.4 µg-eq/cm² for rat and human skin membranes, respectively.
Based on the flux values a human/rat ration of 1:1.2 was calculated for dermal penetration.

In conclusion, micronized FAT 65080, applied to rat and human skin membranes, penetrated at an extremely low rate and to a very limited extent through the skin membranes. The penetration through rat split-thickness skin membranes was slightly higher than through human split-thickness skin membranes. Although, the test item entered the skin membrane after exposure, it did not penetrate through the membrane to a significant extent. The penetration into lower skin layers (below stratum corneum) was more pronounced in rat skin membrane.

Ref.: 12

Comment
FAT 65080 was micronized: The mean particle size of the test item was found to be d(0.5) = 440 nm

Table 7 Recovery of radioactivity following application of [^{14}C]-FAT 65080 (ETH50) to rat skin membranes (Group Q1, Formulation A1)

[% of Dose]									
Dose applied [µg·cm^{-2}]	1912								
Cell No.	1	2	3	4	5	6	7	Mean	SD
Dislodged Dose									
Membrane Rinse	50.33	59.83	57.54	85.49	62.27	86.29	92.27	**70.67**	16.86
Perfusates									
0-24 h	**0.16**	**0.15**	**0.16**	**0.09**	**0.10**	**0.11**	**0.08**	**0.12**	**0.03**
Remaining Dose									
Tape Strips I	25.38	25.62	29.05	6.72	13.94	8.17	2.92	**15.97**	10.60
Remaining Skin Membrane	10.02	4.98	5.85	2.48	9.73	1.50	0.08	**4.85**	3.90
Cellwash	8.65	3.49	2.30	2.21	8.26	1.94	0.19	**3.86**	3.29
Recovery	**94.63**	**94.07**	**94.89**	**98.99**	**94.31**	**98.01**	**96.63**	**95.48**	1.49

Given the large variation in the data, the dermal in vitro absorption value for the rat is set as the mean absorption + 2SDs. The mean absorption is calculated by adding the concentration in the perfusate plus the concentration in the remaining skin membrane. This results in an dermal absorption for the rat of 0.12+ 4.85 + 2x√(0.032 + 3.92) = 12.77%. It should be noted that this is a very conservative value, especially since this value is driven by the large dose remaining on the skin membrane.

Table 8 Recovery of radioactivity following application of [^{14}C]-FAT 65080 (ETH50) to human skin membranes (Group Q2, Formulation A1)

[% of Dose]								
Dose applied [µg·cm^{-2}]	1912							
Cell No.	8	10	11	12	13	14	Mean	SD
Dislodged Dose								
Membrane Rinse	68.92	85.49	83.00	59.76	64.42	77.49	**73.18**	10.41
Perfusates								
0-24 h	**0.04**	**0.02**	**0.14**	**0.12**	**0.20**	**0.11**	**0.10**	0.07
Remaining Dose								
Tape Strips I	25.44	6.46	10.12	17.50	21.62	11.13	**16.38**	7.35
Remaining Skin Membrane	0.31	< 0.01	0.19	0.06	0.19	0.31	**0.18**	0.13
Cellwash	0.20	3.13	2.82	4.77	2.42	4.62	**2.99**	1.67
Recovery	**94.91**	**95.11**	**96.28**	**82.21**	**88.84**	**93.86**	**91.83**	5.38

Given the large variation in the data, the dermal in vitro absorption value for the human skin is set as the mean absorption + 2SDs. The mean absorption is calculated by adding the concentration in the perfusate plus the concentration in the remaining skin membrane. This results in an dermal absorption for human skin of 0.10+ 0.18 + 2x√(0.072 + 0.132) = 0.57%. It should be noted that this is a very conservative value.

In vivo rat dermal absorption study

Guideline: OECD 427
Species/strain: Wistar HanBrl:WIST (SPF) rats
Group size: 4 males per group (one dose, 4 sacrifice time points)
Test substance: Non-radiolabelled FAT 65'080 + [^{14}C]-labelled FAT 65'080, specific activity 2.11 µCi/mg, micronized with surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol) to mixture of 90.88 mg [^{14}C]-labelled FAT 65'080/ml
Batch: Non-radiolabelled: KOC00050/004E

	Radiolabelled: 49336-1-61
Purity:	Non-radiolabelled: 98%
	Radiolabelled: >99%
Particle size:	d(0.5) = 440 nm
Dose level:	Single topical application of 2 mg/cm² (nominal) or 1829 µg/cm² (actual) in 200 µl on 10 cm² shaved, intact skin for 6 hours under semi-occlusive conditions
Route:	topical
Exposure period:	6 hours
Sacrifice time points:	6, 24, 48, or 72 hours after application
Recovery:	92.76%, 94.36%, 97.00%, 95.11%
GLP:	in compliance

The formulated test item was dermally applied at a nominal dose level of 1829 µg/cm² to a skin area of 10 cm². The dermal absorption of the test item during a 6 hour exposure period was determined. Furthermore, the amount remaining in/on the skin at the application site after washing was determined at three additional time points of 24, 48 and 72 hours after application of the test item in order to estimate the depletion of the dose associated with the application site. The association of the remaining test item in/on the skin at the application site was investigated by skin stripping in order to separate the stratum corneum from the epidermis. Urine and faeces were collected up to 72 hours after administration. Residue levels in blood, plasma, liver and kidneys were also measured up to 72 hours after application.
During 6 hours exposure to formulated [Triazine-U-^{14}C] FAT 65080 (ETH50), only 0.11% of the dose was systemically absorbed. The penetration rate was calculated to be 0.3345 µg·cm^{-}2·h^{-}1.
The highest blood and plasma concentrations were found at the end of exposure (6 h), accounting for 0.1272 and 0.2327 ppm FAT 65080 equivalents, respectively. Thereafter the concentration in blood and plasma decreased reaching the limit of quantification within 48 hours. The dermally absorbed FAT 65080 and/or its radiolabelled metabolites were exclusively present in plasma. The highest residues in liver and kidneys were also found 6 hours after start of exposure, accounting for 0.1812 ppm in liver and 0.1051 ppm in kidneys.
The depletion kinetics of the residues was very similar to that observed in blood.
At the end of exposure period 90-92% of the dose could be dislodged from the application site. After the washing procedure, 6 hours after start of exposure, 2.1-4.4% of the dose remained in/on the treated skin area located almost exclusively in/on the stratum corneum.
Less than 0.1% of the dose was found in the lower skin layers. During the 3 days after exposure the remaining amount of FAT 65080 (ETH50) in/on the treated skin after washing lead only to a very low increase of the systemic absorption. The amount totally absorbed accounted for 0.15% of the applied dose, indicating that the remaining amount of FAT 65080 in/on the skin was not available for dermal absorption.
The systemically absorbed test item was slowly excreted with the urine and the faeces. Within 72 hours 0.05% and 0.03% of the dose were excreted with the urine and faeces, respectively.
Total recovery was 92-97% of the applied radioactivity in each subgroup.

Ref.: 13 (subm I)

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral

Guideline:	OECD 408
Species/strain:	Sprague-Dawley, Crl CD® (SD) IGS BR rats
Group size:	10 males + 10 females per dose (6 weeks old at start of study)
Test substance:	FAT 65'080, d(0.5) = <15.4 µm
Batch:	KOC00050/004.E
Purity:	98%
Dose levels:	50, 250 and 1000 mg/kg bw/day in 0.5% carboxymethylcellulose (w/v) in purified water
Route:	oral, gavage
Exposure period:	13 weeks
GLP:	In compliance

Clinical examination revealed piloerection in males treated at 250 or 1000 mg/kg bw/day, which was probably treatment related, but was considered not adverse. The finding was infrequent (1/10 at mid-dose, 3/16 at high-dose) and had always disappeared by week 10.
Other clinical findings included alopecia, differences in body weight and unilateral linear chorioretinopathy, but were considered not treatment related. The changes were minor, occurred at similar incidence in control animals and there was no dose-relationship.

Clinical pathology showed no treatment related effects.

Laboratory investigations revealed slight to moderate differences in white blood cell counts and biochemistry, which were considered not treatment related. Values were within normal ranges and there was no dose-relationship.
On week 6, dose related changes in testosterone, oestrogen and progesterone were observed. The variations were considered to be in relation with oestrous stages (which were not affected by treatment) and therefore to reflect biological variability rather than any treatment-related effect.
There was a dose-related reduction of mean urine volume at 250 (females) or 1000 (females and males) mg/kg bw/day, compared to controls. In the absence of biochemical or histological signs of renal dysfunction, these differences were not considered as biologically relevant.
Due to high inter-individual variations, the changes in white blood cell counts, sexual hormones and urine volumes were not statistically significant.

When measured on day 2 (24 hours after dosing on Day 1) and on weeks 6 and 13 for groups 2, 3 and 4, which received the test item at 50, 250 and 1000 mg/kg bw/day, plasma levels of the test substance were not quantifiable for all animals at any time-point.

When male and female rats were treated with the test substance by daily oral gavage at dose-levels up to 1000 mg/kg bw/day for 13 weeks, followed by a 4-week recovery period, no adverse effects were noted. Therefore, the NOAEL (No Observed Adverse Effect Level) after oral gavage under the experimental conditions of the study was determined to be 1000 mg/kg bw/day.

Ref.: 3 (subm I)

Comment
Piloerection could be a sign of neurotoxicity and, although effects were seen only in a few animals and had disappeared after ten weeks, it should be considered adverse. A functional observation battery was performed (at week 12 only) but this only considers functional effects. The piloerection appears to be an acute effect, and was also seen in the acute oral toxicity study.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity in vitro

Bacterial Reverse Mutation Test

Guideline:	OECD 407 (1997)
Species/strains:	Salmonella typhimurium TA98, TA100, TA102, TA1535 and TA1537 and Escherichia coli strain WP2uvrA
Test substance:	FAT 65'080, d(0.5) = <15.4 µm
Batch:	ETH50/129B
Purity:	>98%
Replicates:	2
Concentrations:	62.5, 125, 250, 500, 1000 µg/plate in DMSO
Test conditions:	Direct plate incorporation method with and without metabolic activation (Aroclor-induced rat liver S9-mix) and preincubation method (60 minutes, 37 ºC) with metabolic activation.
Solubility:	Precipitation of the test substance was found from about 500 µg/plate onward
GLP:	in compliance

Results:
A moderate to marked precipitate was observed in the test dishes when scoring the revertants at dose-levels ≥ 500 µg/plate.
No toxicity was noted towards all the strains used, both with and without S9 mix. The test item did not induce any significant increase in the number of revertants, both with or without S9 mix, in any of the five strains.
Under the conditions of the test, ETH50 did not show mutagenic activity in the bacterial reverse mutation test with Salmonella typhimurium and Escherichia coli.

Ref.: 14 (subm I)

***In vitro* Mammalian Chromosome Aberration Test**

Guideline:	OECD 473 (1997)
Species/strains:	Human lymphocytes
Test substance:	FAT 65'080, d(0.5) = <15.4 µm
Batch:	KOC00050/004.E
Purity:	98.8%
Cell system:	Heparinized whole blood from two healthy donors, added with culture medium containing the mitogen phytohemagglutinin and incubated at 37ºC for 48 hours
Concentrations:	Vehicle: DMSO 1st experiment 3 h exposure, 20 h harvest time, with or without S-9-mix: 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL. 2nd experiment: 20 h exposure, 20 h harvest time without S-9-mix: 15.6, 31.3, 62.5, 125, 250, 500 µg /mL 44 h exposure, 44 h harvest time without S-9-mix: 15.6, 31.3, 62.5, 125, 250, 500 µg /mL

	3 h exposure, 20 h harvest time with S-9-mix: 15.6, 31.3, 62.5, 125, 250, 500 µg /mL 3 h exposure, 44 h harvest time with S-9 mix: 15.6, 31.3, 62.5, 125, 250, 500 µg /mL
Test conditions:	see below
GLP:	in compliance

In the first experiment, lymphocyte cultures were exposed to the test or control items (with or without S9 mix) for 3 hours then rinsed. Cells were harvested 20 hours after the beginning of treatment, corresponding to approximately 1.5 normal cell cycles.
The second experiment was performed as follows: without S9 mix, cells were exposed continuously to the test or control items until harvest, with S9 mix, cells were exposed to the test or control items for 3 hours and then rinsed.

Cells were harvested 20 hours and 44 hours after the beginning of treatment, corresponding to approximately 1.5 normal cell cycles and 24 hours later, respectively. One and a half hours before harvest, each culture was treated with a colcemid solution (10 µg/mL) to block cells at the metaphase-stage of mitosis. After hypotonic treatment (KCl 0.075 M), the cells were fixed in a methanol/acetic acid mixture (3/1; v/v), spread on glass slides and stained with Giemsa. All the slides were coded for scoring. The dose-levels of the positive controls were as follows: without S9 mix, Mitomycin C: 3 µg/mL (3 hours of treatment) or 0.2 µg/mL (continuous treatment), with S9 mix, Cyclophosphamide: 12.5 and 25 µg/mL

Results:
In the culture medium, the dose-level of 500 µg/mL showed a moderate precipitate. A slight to moderate precipitate was observed at the end of the treatment period, generally at dose-levels ≥ 250 µg/mL.

Experiments without S9 mix:
Cytotoxicity:
Following the 3-hour treatment, a slight to marked decrease in mitotic index was noted without any clear evidence of a dose relationship (26-62% decrease). Following the 20-hour treatment, a slight decrease in mitotic index was noted at 500 µg/mL (33% decrease). Following the 44-hour treatment, no decrease in mitotic index was noted at any dose-level.

Metaphase analysis:
No noteworthy increase in numerical aberrations was noted throughout the study.
No significant increase in the frequency of cells with structural chromosomal aberrations was noted after 3-, 20- as well as 44-hour treatments.

Experiments with S9 mix:
Cytotoxicity:
At the 20-hour harvest time in the first experiment, a slight to moderate decrease in mitotic index was noted without any clear evidence of a dose relationship (26-43% decrease). At the 20-hour harvest time in the second experiment, a moderate decrease in mitotic index was noted at 500 µg/mL (45% decrease). At the 44-hour harvest time, a slight to moderate decrease in mitotic index was noted without any clear evidence of a dose relationship (25-39% decrease).

Metaphase analysis:
No noteworthy increase in numerical aberrations was noted throughout the study.
No significant increase in the frequency of cells with structural chromosomal aberrations was noted in either experiments and at either harvest times.

In conclusion: Under the conditions of the test, ETH50 did not induce chromosome aberrations in cultured human lymphocytes. No noteworthy increase in numerical aberrations was noted throughout the study.

Ref.: 15 (subm I)

Mouse lymphoma (TK locus) *in vitro* mutation

Guideline:	OECD 476 (1997)
Species/strains:	mouse lymphoma L5187Y cells
Test substance:	FAT 65'080, d(0.5) = <15.4 µm
Batch:	KOC00050/004.E
Purity:	98.8%
Concentrations:	
	1st experiment 3 h exposure, 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL with or without S9-mix 2nd experiment 24 hour exposure: 6.25, 12.5, 25, 50, 100, and 200 µg/mL, without S9-mix 3 h exposure: 6.25, 12.5, 25, 50, 100, and 200 µg/mL, with S9-mix
Vehicle:	DMSO
Positive controls:	-S9:methylmethane sulfonate (MMS); +S9: cyclophosphamide (CPA)
GLP:	in compliance

For the 3-hour treatment, approximately 0.5 x 106 cells/mL in 20 mL culture medium were exposed to the test or control items, at 37 °C. For the 24-hour treatment, approximately 0.15 x 106 cells/mL in 20 mL culture medium (RPMI 5) were exposed to the test or control items, at 37 °C.

The numbers of mutant clones (differentiating small and large colonies) were checked after the expression of the mutant phenotype.

The cloning efficiencies CE2 and the mutation frequencies of the vehicle and positive controls met the study acceptance criteria and it was therefore considered valid.

Since the test item was poorly soluble and non-toxic in the preliminary assay, the highest dose-level used for treatment in the main test was limited by precipitate in the culture medium.

Experiments without S9 mix: The selected treatment-levels were:

- 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL, for the first experiment (3-hour treatment), and
- 6.25, 12.5, 25, 50, 100 and 200 µg/mL for the second experiment (24-hour treatment).

A slight to marked precipitate was observed at the end of the treatment period at dose-levels ≥ 50 µg/mL. No noteworthy toxicity was noted at any dose-level, in either experiment. The test item did not induce any significant increase in the mutation frequency in either experiment. The ratio of small and large colonies was not adversely affected.

Experiments with S9 mix: The selected treatment-levels were (3-hour treatment)

- 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL, for the first experiment, and
- 6.25, 12.5, 25, 50, 100 and 200 µg/mL, for the second experiment.

A slight to marked precipitate was observed at the end of the treatment period at dose-levels ≥ 50 µg/mL. No noteworthy toxicity was noted at any dose-level, in either

experiment. The test item did not induce any significant increase in the mutation frequency in either experiment.
Under the experimental conditions, ETH50 did not show any mutagenic activity in the mouse lymphoma assay.

Ref.: 16 (subm I)

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data submitted

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Prenatal Developmental Toxicity Study in Sprague-Dawley Rats - Oral Administration (Gavage)

Guideline:	OECD 414
Species/strain:	Sprague-Dawley, Crl CD® (SD) IGS BR
Group size:	24 mated females per dose (10-11 weeks old at beginning of treatment period)
Test substance:	FAT 65'080, d(0.5) = <15.4 µm
Batch:	KOC00050/004.E
Purity:	98%
Dose level:	0, 100, 300, 1000 mg/kg bw/d in 0.5% carboxymethylcellulose in purified water
Route:	Oral (gavage, 5 ml/kg bw/d)
Exposure period:	Day 6 - 19 p.c.
GLP:	In compliance

The test substance administered daily by gavage to pregnant Sprague-Dawley rats from day 6 to day 19 p.c. at dose-levels of 100, 300 or 1000 mg/kg bw/day did not elicit any signs of maternal toxicity.

At 1000 mg/kg bw/day, one foetus presented a mandibular micrognathia associated with soft tissue malformations: microglossia and dilated cerebra. As only one foetus was affected, even though it was in the high-dose group, it was considered not to be related to treatment.
At 300 mg/kg bw/day, two foetuses also presented soft tissue malformations: dilated renal pelvis and/or dilated ureter. As this also occurred in the control group, at higher incidence, this was considered not to be related to treatment.
Some skeletal variations were observed, but these were minor, not dose-related and didn't reach statistical significance and/or were not observed at the high dose-level; they were consequently considered not to be treatment-related.

Consequently, under these experimental conditions, the No Observed Effect Level (NOEL) for both the maternal and the prenatal developmental toxicity is set at 1000 mg/kg bw/day.

Ref.: 11 (subm I)

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Phototoxic and photoallergenic potential by cutaneous route in guinea pigs

Guideline:	/
Species/strain:	Male guinea pigs, Hartley Crl: (HA) BR
Group size:	5 or 10 animals per group
Test substance:	FAT 65'080/A, d(0.5) < 15um
Batch:	- Study plan: none - Labelling: ETH 50/129B
Purity:	> 98%
UV irradiation:	Toxicotronic 312/365 nm (Vilbert/Lourmat, Marne-la-Vallée, France). The lamp consists of two groups of three fluorescent tubes producing either UV A (365 nm) or UV B (312 nm). The irradiation was performed in two stages, first irradiation with UV B and then irradiation with UV A, at an infra-erythematogenic irradiation dose (score of erythema ≤ 0.5). The irradiation doses were approximately 9 joules/cm² for UV A and 0.1 joule/cm² for UV B.
Dose level:	0.1 ml of the test substance in propylene glycol at the concentration of 10% (w/w) on area of 9 cm² (interscapular region).
Groups:	Group 1: irradiation control group (5 animals) Group 2: test item control group (five animals) Group 3: test group (ten animals) Group 4: vehicle control group (five animals)
Route:	Topical
Observation period:	1, 4, 24 and 48 hours after the challenge application and/or irradiation
GLP:	In compliance

The design of the study was based on the method published by Unkovic et al., Sci. Tech. Ani. Lab 8-3: 149-160 (1983).

The photoallergenic potential of the test item was assessed as follows:

- during an induction period of 8 days, six topical applications and/or UV A+UV B irradiation (including that for phototoxic potential assessment) were performed on the anterior scapular area of animals of all groups,
- during a rest period of 20 days, the animals received no treatment and no irradiation,
- on day 29, a challenge phase was performed by topical application and/or irradiation to the posterior area of the right (UV A) and left (UV B) flanks of the animals.

For each treatment, a dose-volume of 0.1 mL of the test item at the concentration of 10% (w/w) in propylene glycol was applied by cutaneous route. The irradiation doses of UV A and UV B were infra-erythematogenic. The cutaneous reactions were evaluated at the treatment sites.
At the end of the study, animals were killed without examination of internal organs. No skin samples were taken from the challenge application sites.

No clinical signs and no deaths were noted during the study. The body weight gain of the treated animals was similar to that of the control animals.

Phototoxic potential
The cutaneous reactions observed at the 1-, 4- and 24-hour readings were questionable to moderate erythema and were of similar incidence in control and treated groups. No cutaneous reactions which could be attributed to a photoirritant effect of the test substance were recorded.

Photoallergenic potential
The cutaneous reactions observed at the 1-, 4-, 24- and 48 hour readings remained in the range of a local reaction at an infra-erythematogenic irradiation dose (questionable to discrete) and were of similar incidence in the control and treated groups. No cutaneous reactions which could be attributed to a photoallergenic effect of the test substance were observed.

Ref.: 7 (subm I)

Comment
Under the experimental conditions, two very specific wavelengths of UV radiation were used without information of the absorption spectra of the substance. Broadband UVA and UVB irradiation would more appropriately mimic the intended use of this cosmetic UV-filter.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

Photo-mutagenicity in Bacteria

Guideline:
Species/strains: Salmonella typhimurium TA 1537, TA98, TA100 and TA 102
Test substance: FAT 65'080, d(0.5) = <15.4 µm
Batch: KOC00050/004.E
Purity: 98%
Replicates: 3 plates per test
Concentrations: 33 – 5000 µg/plate (in acetone)
UV irradiation: Source of light: Xenon-lamp (Sunset CPS, ATLAS) emitting a continuous spectrum of simulated sunlight. UV dose:
GLP:

The test material FAT 65'080 (batch No. KOC00050/004.E; purity 98%) was dosed as a suspension in acetone. The irradiation was performed with a Xenon-lamp (Sunset CPS, ATLAS, D-63558 Gelnhausen) that emits a continuous spectrum of simulated sunlight. The intensity of irradiation was 0.1 – 0.3 mW/cm² and each bacterial strain received its respective amount of tolerable UVA and UVB exposure.
The assay was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate. Metabolic activation with rat S9 was also applied in each experiment. Based on absence of cytotoxicity in the preliminary experiment the test item was dosed at the following concentrations: 33, 100, 333, 1000, 2500, or 5000 µg/plate No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. The plates incubated with the test item showed normal background growth up to 5000 µg/plate in all strains used. No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with FAT 65´080/B under irradiation with artificial sunlight at any dose level. The test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Under the conditions of the test, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Ref.: 17 (subm I)

Photo-clastogenicity assay with V79 Chinese Hamster cells in vitro

Guideline:	OECD 473
Species/strains:	V79 cells derived from Chinese Hamster
Test substance:	FAT65'080, d(0.5) = <15.4 µm
Batch:	KOC00050/004.E
Purity:	98%
Concentrations:	1.3 – 15 µg/mL in tetrahydrofuran
Cell system:	
Light source:	Xenon-lamp (Suntest CPS, ATLAS) with an additional special filter glass emitting visible UVA/UVB light > 290 nm
UV doses:	125 mJ/cm² UVA (Exp. I and II) or 200 mJ/cm² UVA (Exp. II)
Positive controls:	without irradiation: EMS; with irradiation: 8-MOP

The light source was an Atlas Suntest CPS, a xenon burner with an additional special filter glass, emitting visible light and UVA/UVB light (ratio: about 30:1) > 290 nm. Cultures were pre-incubated with the test item for 30 minutes where after the cultures were exposed to 125 mJ/cm² UVA (Exp. I and II) or 200 mJ/cm² UVA (Exp. II). Three hours after start of treatment, the cultures were washed. Corresponding cultures with the test item were kept in the dark for the 3 hrs exposure period. The chromosomes were prepared 18 hrs (Exp. I) and 28 hrs (Exp. II) after start of treatment with the test item.

In the cytogenetic experiments for each experimental group two parallel cultures were set up. Per culture at least 100 metaphase plates were scored for structural chromosome aberrations.

Dose selection for the cytogenetic experiments was performed considering the occurrence of test item precipitation and included test item concentrations between 0.12 and 15 µg/mL (with and without irradiation). For both experiments the concentrations used were 1.3 up to 15 µg/ml and due to precipitation, the endpoints evaluation was based on concentrations of 1.3, 2.5, and 5.0 µg/ml.

In both experiments, no toxic effects indicated by reduced mitotic indices or cell numbers of below 50% of control were observed. In Experiment I and II, in the absence and the presence of irradiation, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item. However, in Experiment I, in the absence of irradiation, two statistically significant increases (2.5% and 2.0%, respectively) were observed, but were within the historical control data range for non-irradiated cultures (0.0–4.0% aberrant cells, exclusive gaps) and, therefore, are regarded as being biologically irrelevant.

In conclusion, it can be stated that under the experimental conditions reported, the test item FAT 65´080/B did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) after irradiation with artificial sunlight when tested up to precipitating concentrations.

Ref.: 18 (subm I)

3.3.11. Human data

No data submitted

3.3.12. Special investigations

***In vitro* androgen receptor binding assay**

Guideline:	/
Species/strains:	cytosolic preparations of prostate gland tissue of Alderley Park rats treated with GnRH antagonist (Antarelix)

Test substance:	FAT 65'080/B, d(0.5) = <15.4 µm
Batch:	KOC0005/004E
Purity:	98%
Concentrations:	vehicle: DMSO positive control: methyl trienolone, $5x10^{-6}$M, $5x10^{-7}$M, $5x10^{-8}$M, $5x10^{-9}$M and 5x10-^{10}M Test substance: $5x10^{-4}$M, $5x10^{-5}$M, $5x10^{-6}$M, $5x10^{-7}$M, $5x10^{-8}$M, $5x10^{-9}$M and $5x10^{-10}$M
GLP:	In compliance

The design of the study was based on the method published by Ashby et al. (2001) Replacement of surgical castration by GnRH inhibition for rat prostrate androgen receptor preparations. J. Appl. Toxicol. 21, 353-354.
Phase I of the study consisted of dosing 8 male Alderley Park rats with the GnRH antagonist (Antarelix) and harvesting the prostate gland cytosol 24 hours later, prior to conducting the in vitro phase of the study (Phase II). In Phase II, cytosolic preparations of prostate gland tissue were incubated with the test substance (FAT 65'080/B) at a range of concentrations (0.5 nM to 0.5 mM) and a fixed concentration (5 nM) of the radiolabelled androgen, methyl trienolone (^{3}H-R1881 (~0.2µCi ≅ $5x10^{-9}$ M R1881) in order to determine the ability of the test substance to displace the ^{3}H-R1881. Preparations were incubated with the test substances for approximately 17 hours at approximately 4 ºC. Quantitation of displacement was used to determine the intrinsic activity of the test substance to interact with the androgen receptor (AR). Two independent assays with duplicates in each assay were performed.
The test substance exhibited no displacement of 3H-R1881 at any of the concentrations tested. Displacement of ^{3}H-R1881 was observed following incubation with increasing concentrations of non-radioactive R1881 (positive control) but not with DMSO (vehicle control). Consistent results were obtained between duplicates within each assay and across both assays.
In conclusion, the inability to displace ^{3}H-R1881 from cytosolic preparations of rat prostate gland tissue, indicated that, at concentrations up to $5x10^{-4}$ M, the test substance does not possess intrinsic potential to interact with the rat androgen receptor in the in vitro androgen receptor binding assay.

Ref.: 8 (subm I)

In vitro estrogen receptor binding assay

Guideline:	/
Species/strains:	cytosolic preparations of uterine tissue of immature Alpk:APfSD (Wistar derived) rats
Test substance:	FAT 65'080/B (technical), d(0.5) = <15.4 µm
Batch:	KOC0005/004E
Purity:	98%
Concentrations:	Vehicle: DMSO Positive control: Estradiol, $5x10^{-6}$M, $5x10^{-7}$M, $5x10^{-8}$M, $5x10^{-9}$M and $5x10^{-10}$M Test substance: $5x10^{-4}$M, $5x10^{-5}$M, $5x10^{-6}$M, $5x10^{-7}$M, $5x10^{-8}$M, $5x10^{-9}$M and $5x10^{-10}$M
GLP:	In compliance

The design of the study was based on the method published by Ashby et al. (2001) Lack of binding to isolated oestrogen or androgen receptors, and inactivity in the immature rat uterotrophic assay, of the ultraviolet sunscreen filters Tinsorb M-Active and Tinsorb S. Regul. Toxicol. Pharmacol. 34, 287-291.

The study consisted of harvesting the uteri from 40 immature Alderley Park rats. Freshly prepared cytosolic preparations of uterine tissue were then incubated with the test substance at a range of concentrations (0.5 nM to 0.5m M) and a fixed concentration (5n M) of the radiolabelled oestrogen, 3H-estradiol in order to determine the ability of FAT 65'080/B to displace the ^{3}H-estradiol. Preparations were incubated with the test substances for approximately 17 hours at approximately 4 ºC. Quantitation of displacement was used to determine the intrinsic activity of the test substance to interact with the oestrogen receptor (ER). Two independent assays with duplicates in each assay were performed.
The results for the test substance showed a good correlation in both assays and between duplicates in each assay, with no increase in radioactivity displacement at the concentrations of test substance tested in both assays.

In conclusion, the inability to displace ^{3}H-Estradiol from cytosolic preparations of rat uterine tissue, indicated that, at concentrations up to $5x10^{-4}$ M, the test substance (FAT 65'080/B) does not possess intrinsic potential to interact with the rat oestrogen receptor in the in vitro oestrogen receptor binding assay.

Ref.: 9 (subm I)

Uterotrophic assay in immature female rats

Guideline:	/
Species/strain:	Immature female Alpk;APfSD rats (Wistar derived)
Group size:	10 females, 19-20 days old at start of experiment
Test substance:	FAT 65'080B, d(0.5) = <15.4 µm
Batch:	KOC0005/004
Purity:	98%
Dose level:	Positive control: Estradiol 0.4 mg/kg bw/day Test substance: 250, 500 or 1000 mg/kg bw/day Daily administration of 10 ml/kg bw in 0.5% carboxymethylcellulose, for three consecutive days
Route:	Oral, by gavage
Exposure period:	Three days
GLP:	In compliance

Groups of ten immature female rats received a single oral dose of 0 (vehicle control), 250, 500 or 1000 mg/kg bw of the test substance once a day for 3 consecutive days. As a positive control, one group of rats received a single oral dose of 0.4 mg β-estradiol/kg bw once a day for 3 consecutive days. The vehicle used for the test substance and β-estradiol was 0.5% carboxymethylcellulose.
The bodyweight of each rat was recorded daily, and detailed clinical observations were made at the same time. At the end of the study (approximately 24 hours after administration of the final dose), all of the animals were killed. The uterus was removed from each animal and trimmed of any fat and adhering non-uterine tissue. The uterus was then opened with a small incision, squeezed and blotted onto filter paper to remove any excess fluid and the uterine wet weight was recorded.

Initial analysis of study dose preparations gave poor results with regard to suspension, low achieved concentration and poor homogeneity. Samples from residues from the animal rooms were analysed and results indicated that the original initial concentrations of these dosing preparations were likely to be correct and that the stability of the dosing preparations was likely to be acceptable.

There were no clinical signs observed during this study for correctly dosed animals. Two animals died soon after dosing, (500 and 1000 mg/kg bw/day) and one animal required euthanasia during the study (1000 mg/kg bw/day). Post-mortem examination revealed dosing accidents to be the cause of death. One animal showed some adverse clinical signs

on day 2 (1000 mg/kg bw/day); on termination examination revealed a dosing accident had occurred.

There was no effect of the test substance on bodyweight or on uterus weight. As expected, oral gavage administration of β-estradiol to the immature rat for three consecutive days resulted in a marked increase in uterine weight, demonstrating a positive uterotrophic response with this substance.
In conclusion, there was no evidence of an uterotrophic response to this test substance.

Ref.: 10 (subm I)

Annex 2: Exposure calculations using ConsExpo

Product
ETH50 Pump Spray (10% ETH50)

Compound

Compound name	ETH50	
CAS number	31274-51-8	
molecular weight	538	g/mol
vapour pressure	4.15E-21	Pascal
KOW	10.4	10Log

General Exposure Data

exposure frequency	75	1/year
body weight	61	kilogram

Inhalation model: Exposure to spray

weight fraction compound	10	%
exposure duration	5	minute
room volume	10	m^3
ventilation rate	2	L/hr
mass generation rate	1	g/sec
spray duration	0.5	minute
airborn fraction	0.2	fraction
weight fraction non-volatile	10	%
density non-volatile	1.5	g/cm^3
room height	2.5	meter
inhalation cut-off diameter	15	micrometer
cloud volume	0.0625	m^3
non-respirable uptake fraction	1	%
Spraying towards exposed person		

Initial particle distribution:

Distribution function	normal	
Median	60	micrometer
s.d.	18	micrometer

Uptake model: Fraction

uptake fraction	1	fraction
inhalation rate	33.3	m^3/day

Dermal model: Direct dermal contact with product: instant application

weight fraction compound	10	%
exposed area	1,75E4	cm^2
applied amount	18	gram

Uptake model: fraction

uptake fraction	0,06	%

Output

Inhalation (point estimates)

inhalation mean event concentration	0,32	mg/m^3
inhalation mean concentration on day of exposure	0,00111	mg/m^3
inhalation air concentration year average	0,000228	mg/m^3/day

inhalation acute (internal) dose	0,000607	mg/kg
inhalation chronic (internal) dose	0,000125	mg/kg/day
Dermal: point estimates		
dermal load	0,103	mg/cm²
dermal external dose	29,5	mg/kg
dermal acute (internal) dose	0,0177	mg/kg
dermal chronic (internal) dose	0,00364	mg/kg/day
Oral non-respirable: point estimates		
oral external dose	0,000443	mg/kg
oral acute (internal) dose	0,000443	mg/kg
oral chronic (internal) dose	9,09E-5	mg/kg/day
Integrated (point estimates)		
total external dose	29,6	mg/kg
total acute dose (internal)	0,0188	mg/kg
total chronic dose (internal)	0,00385	mg/kg/day

Ref.: 4 (Sub. VI)

Product

ETH50 Propellant Spray (10% ETH50, 40% Propane/Butane)

Compound		
Compound name	ETH50	
CAS number	31274-51-8	
molecular weight	538	g/mol
vapour pressure	4,15E-21	Pascal
KOW	10.4	10Log
General Exposure Data		
exposure frequency	75	1/year
body weight	61	kilogram
Inhalation model: Exposure to spray		
weight fraction compound	10	%
exposure duration	5	minute
room volume	10	m³
ventilation rate	2	L/hr
mass generation rate	1	g/sec
spray duration	0.5	minute
airborn fraction	0.5	fraction
weight fraction non-volatile	10	%
density non-volatile	1.5	g/cm³
room height	2.5	meter
inhalation cut-off diameter	15	micrometer
cloud volume	0.0625	m³
non-respirable uptake fraction	1	%
Spraying towards exposed person		
Initial particle distribution:		
Distribution function	Normal	
Median	50	micrometer
s.d.	15	micrometer

Uptake model: Fraction

uptake fraction	1	fraction
inhalation rate	33.3	m³/day

Dermal model: Direct dermal contact with product : instant application

weight fraction compound	10	%
exposed area	1.75E4	cm²
applied amount	18	gram

Uptake model: fraction

uptake fraction	0.06	%

Output

Inhalation (point estimates)

inhalation mean event concentration	1.26	mg/m³
inhalation mean concentration on day of exposure	0.00437	mg/m³
inhalation air concentration year average	0.000897	mg/m³/day
inhalation acute (internal) dose	0.00238	mg/kg
inhalation chronic (internal) dose	0,000489	mg/kg/day

Dermal: point estimates

dermal load	0.103	mg/cm²
dermal external dose	29.5	mg/kg
dermal acute (internal) dose	0.0177	mg/kg
dermal chronic (internal) dose	0,00364	mg/kg/day

Oral non-respirable: point estimates

oral external dose	0.00127	mg/kg
oral acute (internal) dose	0.00127	mg/kg
oral chronic (internal) dose	0.000261	mg/kg/day

Integrated (point estimates)

total external dose	29.6	mg/kg
total acute dose (internal)	0.0214	mg/kg
total chronic dose (internal)	0.00439	mg/kg/day

Ref.: 5 (Sub. VI)

Product

ETH50 Propellant Spray (10%), particles after drying

Compound

Compound name	ETH50	
CAS number	31274-51-8	
molecular weight	538	g/mol
vapour pressure	4.15E-21	Pascal
KOW	10.4	10Log

General Exposure Data

exposure frequency	75	1/year
body weight	61	kilogram

Inhalation model: Exposure to spray

weight fraction compound	10	%

exposure duration	5	minute
room volume	10	m^3
ventilation rate	2	1/hr
mass generation rate	1	g/sec
spray duration	0.5	minute
airborn fraction	0.5	fraction
weight fraction non-volatile	30	%
density non-volatile	1.5	g/cm^3
room height	2.5	meter
inhalation cut-off diameter	15	micrometer
cloud volume	0.0625	m^3
non-respirable uptake fraction	1	fraction
Spraying towards exposed person		

Initial particle distribution:

Distribution function	Normal	
Median	37	micrometer
s.d.	12	micrometer

Uptake model: Fraction

uptake fraction	1	fraction
inhalation rate	33.3	m^3/day

Dermal model: Direct dermal contact with product: instant application

weight fraction compound	10	%
exposed area	1.75E4	cm^2
applied amount	18	gram

Uptake model: fraction

uptake fraction	0.06	%

Oral model: Oral exposure to product:

weight fraction compound	10	%

Uptake model:

Output

Inhalation (point estimates)

inhalation mean event concentration	5.47	mg/m^3
inhalation mean concentration on day of exposure	0.019	mg/m^3
inhalation air concentration year average	0.0039	mg/m^3/day
inhalation acute (internal) dose	0.0104	mg/kg
inhalation chronic (internal) dose	0.00213	mg/kg/day

Dermal: point estimates

dermal load	0.103	mg/cm^2
dermal external dose	29.5	mg/kg
dermal acute (internal) dose	0.0177	mg/kg
dermal chronic (internal) dose	0.00364	mg/kg/day

Oral: point estimates

oral external dose	-	mg/kg
oral acute (internal) dose	-	mg/kg
oral chronic (internal) dose	-	mg/kg/day

Oral non-respirable: point estimates

oral external dose	0.153	mg/kg
oral acute (internal) dose	0.153	mg/kg
oral chronic (internal) dose	0.0314	mg/kg/day

Integrated (point estimates)

total external dose	29.7	mg/kg
total acute dose (internal)	0.181	mg/kg
total chronic dose (internal)	0.0372	mg/kg/day

Ref.: 1 (Sub. VI)

The Chemical Company

CORRECTED SUPPLEMENT III

Corrected January 2011

TO THE TOXICOLOGY SUMMARY
And
SAFETY ASSESSMENT FOR ETH50 (FAT 65'080) as UV FILTER in SUNSCREEN PRODUCTS

(First Dossier Submitted on 08 November 2005)

Original Supplement III submitted by Ciba Inc.
Basel, Switzerland; 13 August 2008

BASF SE
07 January 2011

CONTENTS

INTRODUCTION

Background to Corrected Supplement III. This corrected version of our Supplement III is provided in order to make the changes needed to the original document since discovery of an error in the calculation of particle size and number parameters for the micronized ETH50. The corrected parameters are shown in Table 4 on page 7 of this document. Consequent to this error, the new values resulted in a 10^6 reduction in particle exposures as calculated for the various studies summarized in this Supplement.

In addition, we have revised the concentration values in the acute inhalation toxicity study following the discussions in the meeting with DG SANCO and members of the nano Expert Working Group in July 2010.

Where corrections or additions to the original document have been made, the corrected text has been underlined throughout this present document.

Backgound to the Original Supplement of additional toxicology information.
In two earlier supplements submitted in November and December 2006, Ciba presented information on the importance of the small particle size to the Ultraviolet light absorbing efficacy of the active ingredient ETH50 (FAT 65'080), on the micronization process used to prepare the end product to be placed on the market, and on the implied safety of the micronized form of the active ingredient.

In a subsequent meeting with the Working Group evaluating our dossier, Ciba agreed to submit additional non-clinical toxicology information derived from studies with the micronized formulation of ETH 50, representative of our product Tinosorb®A2B, which is the material that would be supplied to sunscreen manufacturers and incorporated into sunscreen formulations. This Supplement presents those data and study results from tests of general acute toxicity, repeated oral gavage dosing with an assessment of developmental toxicity, percutaneous penetration in vitro, in vivo oral ADME with large particle sized ETH50 ($d_{0.5}$ ~6 µm), dermal ADE with micronized ETH50 [$d_{0.5}$ ~80 nm], and an inhalation acute study with evaluation of the pro-inflammatory response in rats. In departure from our usual regulatory hazard evaluation program, induced because of the high interest in micronized UV filters containing particles in the nanometer size range, we have used our formulated micronized product and conducted a 13-week phototoxicity study in hairless mice and a 13-week dermal dosing study with rats, in addition to 2 in vivo genotoxicity studies (mouse micronucleus; rat liver UDS).

Completion of this Supplement has suffered unforeseeable delays in execution of some parts of the analytical chemistry associated with these studies. All but one have been resolved satisfactorily. The remaining issue is that at least four of these final reports carry a notice of exclusion from GLP-compliance for the analytical chemistry results. The affected reports are as follows: 1) repeated oral gavage dosing with an assessment of developmental toxicity, 2) 13 week rat dermal toxicity test, 3) in vivo mouse micronucleus assay, and 4) in vivo rat liver UDS assay. The contracted testing laboratory, CIT, Evreux, FR, failed a GLP compliance inspection that noted inadequacies in a number of SOPs associated with toxicology study reports, including bio-analysis and dosage form analysis. The

laboratory has revised their SOPs, has gained re-certification from the French authority, and is in the process of re-conducting the affected study phases so as to bring the supporting analytical work, mainly dose formulation analysis, into full compliance. Upon completion of these activities at CIT a final report amendment will be issued for each affected study.

Ciba has not yet received a delivery schedule for revision of our affected reports, but we do not anticipate changes in the studies' conclusions. Therefore, we ask the Working Group's understanding of this situation and that they move forward with the review of our dossier. We will provide the amended final reports as soon as possible.

1. CHARACTERISATION OF THE INGREDIENT

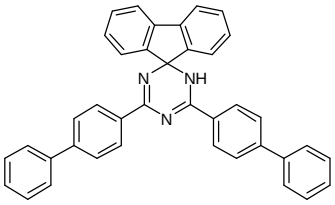
1.8. Purity, composition, particle size distribution and substance codes.
1.8.1. Analytical Profile and by-products in ETH50 Used for toxicity testing

Six additional different batches (Lots) of ETH50 have been used for studies presented in this third Supplement.
The purity was determined by HPLC with an external-standard method, and ranges from 97.2% (w/w) to 98.5% (w/w) expressed as active molecule. The by-product profile of these batches are comparable and are in total <1% area. Biphenyls were not detected (detection limit < 0.01%). The content of xylene was determined by GC and ranged from 0.7% to 2%. **(Ref. A; Ref. B).**

Purity of the batches used for the toxicological assays is summarized in the Table 1 below.

Table 1. **Test Substance Denomination**	**Batches (Lots)**	**Measured purity active (% w/w)**
FAT 65080/D(*) ETH50= Trisbiphenyltriazin	Lot 11103Cl4AA	98.4 ± 2
	Lot 11104Cl4AA	98.5 ± 2
	Lot 11105Cl4AA	98.4 ± 2
	Lot 11106Cl4AA	98.5 ± 2
FAT 65080/E – C-801 [C-801/26 lot 5/50; KRG328-2 (Lot 04122FC7)(micronized)	Lot 11106CL4AA	98.5 ± 2
FAT 65080/F(**) (Lot 04122FC7)(micronized)	Lot 37874FC6	97.2 ± 2
	Lot 37875FC6	97.4 ± 2
(*) Mixture of 4 batches (Lots) produced within one production campaign according to the same production procedure. (**) Active is a mixture of 2 batches (Lots), produced within one production campaign according to the same production procedure.		

Analytical Profile and by-products in ETH50 Used for toxicity testing
A revised analytical method using LC-MS for the analysis of ETH50 in plasma samples was developed to give better detection sensitivity and as a result new information has become available regarding by-products of ETH50 synthesis present in the various samples of test material used to support our safety dossier. This by-product is formed during the synthesis process and has been part of each tested batch of ETH50 **(Ref. C)**. This isomer is discussed further in the UDS Assay summary section and in the discussion section of this dossier. A recent re-analysis of the ETH50 batches has been conducted; the results for the isomer concentrations are summarized in the following Table 2.

Table 2. Test Substance Denominations **(Batches)**	**Relative Ratio** (% Isomer as ETH50 peak area)	**By-Product Found**
FAT 65080/B (KOC0050/004E)	0.008%	 Molecular Weight =537.67 Exact Mass =537.22 Molecular Formula =C39H27N3
FAT 65080/D (Mixture of 4 Lots: 11103CL4AA; 11104CL4AA; 11105CL4AA; 11106CL4AA)	0.025%	
FAT 65080/E KRG 238.2 Liq. (Lot 11106CL4AA micronized)	0.017%	
FAT 65080/F (Lot 04122FC7 – mixture of Lot 37874FC6 and Lot 37875FC6) (micronized - Tinosorb A2B)	<0.003%	4',6'-Bis-biphenyl-4''-yl-1'2'-dihydro-spiro[9H-fluorene-9,2'-[1,3,5]triazine]

1.8.2. Test Articles Used and Particle Size Characterization

Particle Size Determination and Appearance

The additional studies included with this dossier were conducted with micronized ETH50 in particle size ranges as summarized in Table 3. In several studies the not micronized form of ETH50 was also used in separate animal groups as a reference point or bridge to our earlier study results; these results provided a comparison for evaluating if the smaller sized particles changed the study's results and outcome.

Table 3. Summary of ETH50 batches and their particle size distributions used in the additional studies submitted.

FAT 65'080 Suffix Used	**Batch number**	**Particle size distribution***	**Comment**
/B	KOC00050/004.E (purity 98%)	♦ 5% < 1.3 µm , ♦ 10% < 2.4 µm ♦ 19.49% < 5 µm 50 % < 15.4 µm. (MMD)	Not micronized
/E	KRG328-2 (Purity 51.4% ETH50) (from LOT11106CL4AA)= MUS/KRG328-2	d(0.5) 81nm d(0.9) 157 nm	This d(0.5) is a worst case distribution compared to the d(0.5)= 100-110 nm of the product's specifications.
/F	Lot 04122FC7(purity 47.6% ETH50 .) from ETH50 lot 37874FC6 & lot37875FC6	d(0.5) 109 nm d(0.9) 175 nm	This d(0.5) is within the expected range for the product's specifications.

***** Particle size distribution measured by FOQELS (Fiber Optic Quasi Elastic Light Scattering)

Radioalabelled ^{14}C-ETH50 was used for the percutaneous penetration in vitro tests and for the ADME and ADE in vivo rat studies. The dosing formulations used radioactive ETH50 mixed with cold ETH50 from batch KOC00050/004.E and micronized at the testing laboratory (RCC) according to Ciba's specifications. In the ADME study (RCC 2007-c) the micronization process yielded only 6 µm

median diameter particle size because the mill was not sufficiently efficient. In subsequent studies, Ciba personnel and lab-scale mill were used to conduct the micronization at RCC and the produced formulation evaluated and characterized according to Ciba's routine methods. The report is included as Appendix II.

At the request of the Working Group, we also provide electron microscope images of the micronized ETH50 formulation. They are included in the report found in Appendix II.

Particle Dosing Estimation

The physical characterization of the particles present in micronized ETH50 was further defined to allow an estimation of the test system dosages calculated as number of particles and surface area of particles administered. These parameters are suggested as the more representative exposure estimate than mass of test item applied. The physical characterization parameters are summarized in the nearby Table 4.

Table 4. Particle parameters for micronized ETH50

Parameter	**Value***
Assumptions	1. 30% concentration of monodisperse particles of size d(0.5); 2. Values are representative for other ETH50 micronized dispersions used in toxicology studies.
d(0.5)	8.7E-08 m
Surface Area	2.38E-14 m^2/particle
Volume	3.45E-22 m^3
Density	1256 kg/m^3
Number particles per cm^3	**2.9 E15**
Estimated Weight of one particle	**4.33E-16 g**
Number particles per g	**2.31E15**
Specific Surface Area	**54.9 m^2/g**

***** Values prepared and summarized by Herzog, B. and Giesinger, J.; Ciba internal report 12 October 2007; **Corrected 07 January 2011**.

Calculation of particle dosages or exposures calculated with these parameter values are assumed to be representative of the various batches of micronized ETH50 used in the toxicology studies reported herein. This is an conservative assumption because only one batch of micronized test item approximated this median particle size; the other batches had a larger median particle size as summarized above in Table 3.

In the following study summaries, the particle doses are derived from the values shown in Table 4. For several of the studies, the particle size distributions were not available until after the study had been completed so the final report does not contain this information.

2. Toxicology Study Summaries

2.1 Acute toxicity - Oral

The acute oral toxicity of micronized ETH50 was evaluated in rats according to OECD (No. 423, 17th December 2001) and EC (2004/73/EC, B.1 tris, 29th April 2004) guidelines. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations **(Ref. 1**).

The test article was from lot number KRG328-2 prepared from ETH50 LOT11106CL4AA [FAT 65'080/E]; purity was 49.5% of the active UV filter ETH50 and contained all excipients used in the product final mixture Ciba places on the market. The particle size distribution of the test item is not stated in the final report but it was measured after this study was completed. Results give a characterization of d(0.5): 81 nm and d(0.9): 157 nm determined by FOQELS technique (Fiber Optic Quasi Elastic Light Scattering).

Methods

The test item was prepared in purified water and was administered by oral gavage under a volume of 10 mL/kg body weight to 2 groups of three fasted female Sprague-Dawley rats. Mortality did not occur in any of the first 3 females dosed with 2000 mg test item/kg body weight so the second group of 3 females received the same dose. Dose is expressed as test item and is not corrected for the 49.5% of active ingredient ETH50.
All dosed animals were observed up to 14 days after dosing for clinical signs, mortality, and body weight gain and then subjected to necropsy. The interpretation of results was based on the classification criteria laid down in Council Directive 67/548/EEC (and subsequent adaptations).
Equivalent doses for particles are as follows:

Dose as mg/kg b.wt	Total particles dosed (number)	Surface area of particles dosed (m^2)
2000	$5.8 \times 10^{+14}$	13.7

Results

Mortality and clinical signs of toxicity did not occur during the study in any of the animals. However, when compared to CIT historical control data, a slightly lower body weight gain was noted in 1/6 females between day 1 and day 8, but it returned to normal thereafter; in another female the body weight gain between day 8 and day 15 was reduced but it remained within the range for historic control females.
The overall body weight gain of the other animals was not affected by treatment with the test item. At necropsy, no apparent abnormalities were observed in any animals.

Conclusions. *For micronized ETH50 the oral LD_{50} is greater than 2000 mg/kg body weight, or greater than 1000 mg a.i./kg body weight, according to this study's results. This dose is equivalent to about 5.8E+14 particles with an approximate total specific surface area of 13.7 m^2 administered to the test system.*

2.2 Dermal Absorption/Percutaneous penetration

2.2.1 In vitro percutaneous penetration, rat & human skin

The absorption and distribution of FAT 65'080 was evaluated in rat and human split thickness skin membranes following the OECD guideline (Guideline for Testing of Chemicals, Skin Absorption: In Vitro Method Guideline 428, April 13, 2004) **(Ref. 2)**. The experiments were performed to determine the extent and rate of penetration through rat and human skin membranes and to compare the penetration rates to support extrapolation of rat study results to the human safety assessment. The

micronized, small particle size of the test article will allow direct extrapolation to the marketed product to which humans would be exposed.

Non-radiolabeled FAT 65'080 (FAT 65'080/B; batch KOC00050/004E) had a purity of 98 %. The [^{14}C] labeled FAT 65'080 was synthesized by ABC Laboratories (Columbia, MO, USA) as [Triazine-U-^{14}C] with a radiochemical purity ≥99% and specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg), and labeled batch number 49336-1-61. The radiochemical was repurified at the testing laboratory, RCC, before use to meet the above specifications.

The dosing suspension was prepared as a mixture of non-labeled and labeled test material to give [^{14}C]-labeled FAT 65'080 with a final specific radioactivity of 40 kBq/mg (1.07 µCi/mg). After removing residual solvent from the mixture it was micronized in a micro-mill with additions of a surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol) using the Ciba-patented process that is used to prepare the commercial product sold for formulation of consumer sunscreens. Samples of the dosing formulation were analyzed for particle size. The micronization milling and particle size distribution were conducted by technical experts from Ciba.

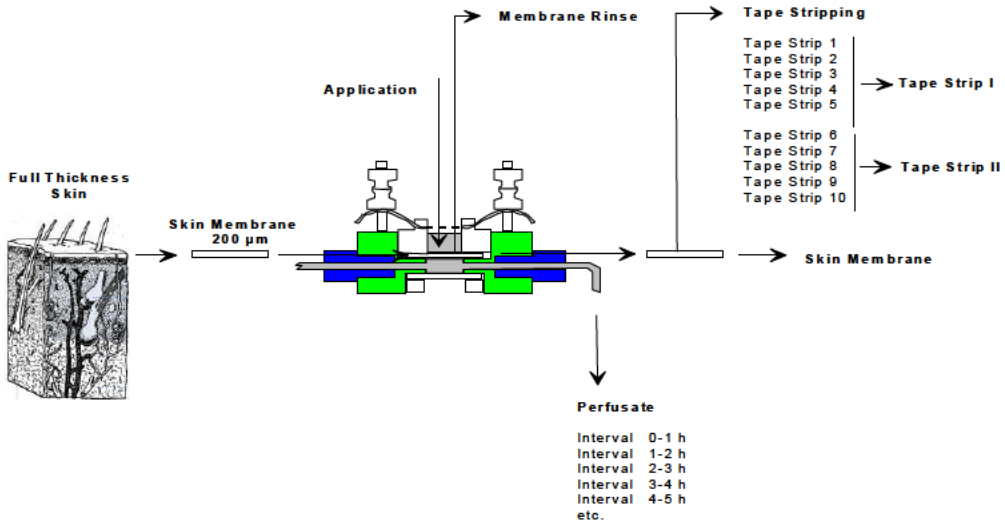
Full thickness skin was removed from 2 male rats (HanBrl: WIST (SPF)) and stored frozen until prepared for use. Human full thickness skin was obtained post-mortem from the dorsal upper leg of 2 individuals and stored frozen until use. Skin membranes of each species were prepared by removing subcutaneous fat from the full thickness sections and then from the stratum corneal aspect removing the upper 200 µm by dermatome. The membranes were then cut into pieces (ca. 1.8 x 1.8 cm) and mounted in flow-through diffusion cells each consisting of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm^2. From each species 7 membranes in cells were prepared and the cells placed in 2 separate manifolds (one with rat, one with human membranes) and connected to a peristaltic pump. For an equilibration period of 0.5 - 1 hour, saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 mL/h.

The integrity of each skin membrane was determined by applying 50 µL tritium water (about 200,000 dpm) to the skin membrane surface and occluding the donor chamber with adhesive tape. The cumulative penetration was determined over 6 hours by collecting hourly fractions. The permeability coefficient (K_p) of each skin membrane was calculated for the 3 - 6 hours interval. Rat skin membranes with $K_p > 3.5 \cdot 10^{-3}$ cm/h and human skin membranes with $K_p > 2.5 \cdot 10^{-3}$ cm/h were excluded from the subsequent experiment. After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

A target dose level of 2 mg/cm² was selected for both rat and human skin based on the topical application rate of final sunscreen formulations assumed to be used by humans. A 13 µL aliquot of the dosing solution, adjusted to pH 6.5 with 20% citric acid solution, was applied manually to each skin membrane preparation using a µL-syringe. The amount applied to each cell was shown to be 1224 µg/cell or 1912 µg/cm^2 by determination of the radioactivity content of three control doses taken prior to the first, in the middle, and after the last administration for each dose level.

The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions. The very low solubility of FAT 65'080 in usual substances led to use as receptor fluid (perfusate) of 6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v); it was delivered at a flow rate of about 3 mL/h during the testing period. Solubility was 0.16 g FAT 65'080/ml perfusate. The perfusate from each cell was collected separately at ambient temperature in 1-hour intervals for the 0 to 6 hour period (6 intervals), and in 2-hour intervals for the remaining exposure period (9 intervals).

The study design is illustrated in the following figure taken from the study report.



Twenty-four hours after application the perfusate sampling was terminated and each skin membrane surface rinsed three times with about 0.5 mL shower gel (1%) in water and then with 0.5 ml tetrahydrofuran for each chamber. All skin membrane rinse fractions were combined according to the individual cells. The skin membranes were removed from the diffusion cell and consecutively stripped until the stratum corneum was removed from the skin membrane, which was 6 to 8 strips per membrane. Up to five consecutive stripping tapes were combined into one specimen and aliquots were measured for radioactivity after mixing with tissue solubilizer (Solvable). The skin membranes remaining after stripping were digested in Solvable and the radioactivity was determined by LSC. The diffusion cells were then washed with 150 mL chloroform and the radioactivity in the cell wash was determined by LSC.

Results. The dosing mixture was shown to have a radiochemical purity of >99%, determined by HPLC at the time of application and a concentration of 95.64 mg [^{14}C]-FAT 65808/mL determined by LSC. Mean particle size, ($d_{0.5}$), was 92 nm and 81 nm in the two measurements taken. The test item was shown to remain stable during the exposure period as indicated by the >98% radiochemical purity determined by HPLC analysis of the skin membrane rinses collected separately from each species. Each of the 7 rat skin membranes and 5 of 7 human skin membranes demonstrated acceptable permeability coefficients and were used for the study. The applied dose was determined to be 1971µg/cm^2 for rat skin and 1912 µg/cm^2 to human skin membranes. Equivalent particle exposures are shown in the adjacent table.

Dose as µg/cm^2	Total particles dosed (number/cm^2)	Surface area of particles dosed (m^2/cm^2)
1971 (human)	4.6 x 10^{12}	0.11
1912 (rat)	4.4 x 10^{12}	0.11

Based on test item found in perfusate the percutaneous penetration rate in rat and human skin was very low or below reliably quantifiable concentrations as shown in the following table.

RCC No B23624	Rat Skin Membrane		Human Skin Membrane	
Applied Dose [µg/cm^2]	1971.7		1912	
Applied Volume [µL]	13		13	
Application Area [cm^2]	0.64		0.64	
Concentration [mg/cm^3]	97		94	
Penetration within	% of dose	µg/cm^2	% of dose	µg/cm^2
6 h	< 0.01	*0.193	< 0.01	*0.150
12 h	0.02	*0.321	< 0.01	*0.246
24 h	0.02	*0.486	0.02	*0.373
Flux[µg/cm^2/h]- measured	0.035		0.026	
Flux[µg/cm^2/h]- estimated$^+$	0.044		0.042	

* value calculated from the measured dpm, most of which are below LQ of 0.04 µg-equivalents

+ estimated by replacing <LQ values with the LQ and calculating flux.

Considering the solubility of FAT 65080 in the perfusate (0.16 µg/mL), the flow rate of the perfusate (about 3 mL/h), and the exposed skin membrane area (0.64 cm) the limit for penetration rate due to the solubility of the test item in the perfusate was estimated to be 0.750 µg/cm^2/h. This indicated the perfusate solubility would not be expected to limit the movement of test item through the skin. We do not interpret our study's results to imply that the measured active ingredient is present as particles as we do not have evidence to support this perspective.

The estimated Flux for rat skin was 0.044 µg/cm^2/h, defined as the penetration rate at steady state between 1-6 hours and calculated by using the corresponding LQ values instead of the measured values, most of which were below LQ. Similarly, the Flux for human skin was estimated to be 0.042 µg/cm^2/h for 1-6 hours based on the LQ values.

(RCCB23624)	**Recovery [% of Dose]***	
Skin Membrane:	**Rat**	**Human**
Applied Dose [µg/cm^2]	1971	1912
Perfusates	0.02 (0.03)#	0.02 (<0.01)#
Remaining Skin membrane	1.36 (1.7)	0.04 (0.07)
Total Absorbed (%)	**1.38**	**0.06**
As µg a.i./cm^2	**27.2**	**1.2**
Skin membrane Rinse	82.73 (8.1)	94.49 (4.3)
Tape Strips	13.46 (4.84)	3.73 (2.19)
Diffusion cell wash	0.64 (0.16)	0.66 (0.43)
Recovery	**98.2** (2.29)	**98.94** (3.04)

* Values are mean (± standard deviation)

Calculated from measured dpm values, most of which were below LQ of about 0.04 µg a.i. equivalents.

The distribution and recovery of the labelled test item at test termination is summarized in the nearby table.

The very low absorption and percutaneous penetration of the test item allowed only an estimation of a flux parameter because the majority of the values were below limits of quantification for the samples. The rat skin was somewhat more permeable to the test item and showed similarly larger amounts of test item in the tape strips compared to human skin membranes, whereas the human skin showed almost 95% removable in surface wipe and membrane rinses. Both test systems showed recovery of more than 98% of applied dose and the test item was shown to remain stable during the 24-hour exposure period.

Based on these results, the test item with particle mean diameters of 92 nm to 81 nm did not penetrate through the skin membranes to a significant extent and, as will be discussed in another section of this dossier, the smaller particles showed a lower tendency than did large particles to permeate skin or deliver the a.i. to the presumed systemic circulation.

2.2.2 In vivo oral absorption, distribution, and elimination- rat

The in vivo absorption, distribution, and elimination of micronized (d(0.5) 92 nm or 81 nm), radiolabelled FAT 65'080 was evaluated in male Han-Wistar rat according to OECD guideline number 417 (Toxicokinetics; April 1984) **(Ref. 3)**. Previous experience with ETH50 indicated very low bioavailability so in this study metabolism was not expected and was not included in the study design. The experiment was performed over 96-hours after a single oral gavage dose to achieve the following 4 objectives:

1) to estimate for the oral route the rate and extent of intestinal absorption of the test item,
2) to investigate the blood kinetics,
3) to determine the pattern of tissue distribution of the test item, and
4) to determine the rates and routes of excretion of the test item.

These results will facilitate interpretation and evaluation of results from our other non-clinical tests with oral gavage dosing, indicate bio-availability of the test item, and because dosing was with the micronized, small particle size of the test article will facilitate extrapolation of the human health safety assessment directly to the marketed product to which humans would be exposed.

This study was conducted immediately after the in vitro percutaneous penetration study described above (RCC B23624) and used the same test item and dosing mixtures. Non-radiolabeled FAT 65'080 (FAT 65'080/B; batch KOC00050/004E) had a purity of 98 %. The [^{14}C] labeled FAT 65'080 was synthesized by ABC Laboratories (Columbia, MO, USA) as [Triazine-U-^{14}C] with a radiochemical purity ≥99% and specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg), and labeled batch number 49336-1-61. The radiochemical was re-purified at the testing laboratory, RCC, before use to meet the above specifications.

The dosing suspension was prepared as a mixture of non-labelled and labelled test material to give [^{14}C]-labelled FAT 65'080 with a final specific radioactivity of 40 kBq/mg (1.07 µCi/mg). After removing residual solvent from the mixture it was micronized in a micro-mill with additions of a surfactant (Plantacare®2000 UP), thickener (xanthan gum) and emulsifier (propylene glycol) using the Ciba-patented process that is used to prepare the commercial product sold for formulation of consumer sunscreens. In support of this study, Ciba provided to RCC the personnel and equipment needed to make this preparation. An aliquot of 0.8 mL was diluted in 2.4 mL purified water to prepare the oral gavage mixture dosed at 1.0 mL per animal. Samples of the dosing formulation were analyzed for particle size and indicated the d(0.5) = 92 nm and 81 nm for the two samples. Equivalent doses as particles are shown in the nearby table.

Dose as mg a.i./kg b.wt	Total particles dosed (number)	No. Particles/kg body wt.	Surface area of particles dosed (m^2)
113	5.22E+13	2.61E+14	1.24
117	5.40E+13	2.70E+14	1.28

The test system was 4 male HanRcc:WIST (SPF): Wistar rats, outbred, SPF-quality of approximately 200 g body weight corresponding to about 8 weeks of age. An acclimatization period was at least five days to the laboratory environment and included one day to the metabolism cages; during acclimatization the animals were kept in groups of 2 rats under conventional hygienic conditions in Makrolon cages with standard soft wood bedding. The animals were kept in rooms maintained at standard conditions, i.e. a temperature of 22±3°C, a relative humidity of 40-70 % and a 12 hours light/dark cycle.
Dosing of the suspension of test item was by gastric intubation at target volume of 0.5 ml per 100 g rat body weight and a nominal dose 100 mg test item per kg body weight. The exact radioactivity

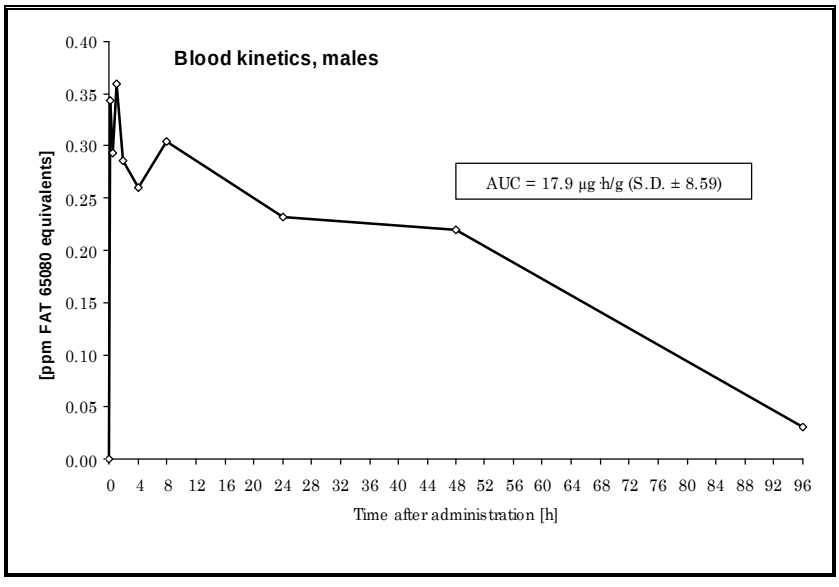
administered was determined via weighing of the application device before (with administration solution) and after administration (empty). The amount of applied test item was calculated based on the concentration of [^{14}C] FAT 65080 in the administration solution. The radiochemical purity of the test item was checked by HPLC at the time of the application.

Urine and feces samples for each of four 24-hour periods were collected individually and separately per metabolism cage; urine was collected into containers on dry ice, feces at room temperature and the daily collections stored frozen until analysis. Study termination at 96-hours after dosing was by CO_2 anesthesia and exsanguinations, during which blood was collected from each animal and separately centrifuged to obtain plasma samples for analysis of radioactivity by LSC.

Cages were rinsed separately with water/ethanol (1:1 v/v) and the rinsates analyzed for collected radioactivity. From each animal, in addition to the blood and plasma collected, samples of each of the following were taken and weighed: liver, kidney, renal fat, muscle; the remaining carcass was retained and processed for determination of radioactivity. Determination of radioactivity was made by LSC after the collected tissues, blood and organs were solubilized by addition of Solvable®, except for aliquots of urine, plasma, and cagewash, to which scintillation mixture was added and then the samples counted.

Results. All 4 animals survived the study period, gained weight, and did not show signs of toxicity or adverse effects. Administered doses ranged from 113 to 117 mg ETH50 /kg body weight with mean of 115.5 mg/kg and 912.98 kBq/animal. The test item purity was 98.2% determined by HPLC analysis of the dosing mixture.

Absorption into systemic circulation was very low based on urinary excretion and was 0.06 % of applied dose. The maximum concentration level in blood was achieved 1 hour after administration, accounting for 0.360 µg FAT 65080 equivalents/g. This concentration level remained almost constant until 8 hours post dosing. Thereafter the concentration decreased with a terminal half-life of about 31 hours. The AUC values, being an index of bioavailability, were calculated to be 17.9 µg·h/g for blood. The nearby figure shows the time course for the radioactivity administered with the test item.



Blood and plasma did not show differences in distribution of the test item. In fact, radiolabelled test item did not exceed LOQ in any tissue or organ at 96-hours after dosing; the LOQ was about 0.091 µg-equivalents per gram except for fat, which was 0.182 µg-equivalents/g. Only remaining carcass, LOQ of 0.012 µg-equivalents/g, showed measurable quantities of test item that approximated 0.07 (± 0.01)% of applied dose.

(RCC B23613)		Excretion
Urine	Time period	**[% of dose]**
	0 – 24 h	**0.04**
	24 – 48 h	**0.01**
	48 – 72 h	**<0.01**
	72 – 96 h	**<0.01**
	Subtotal	**0.06**
Feces		
	0 - 24 h	**92.27**
	24 - 48 h	**1.06**
	48 - 72 h	**<0.01**
	72 - 96 h	**<0.01**
	Subtotal	**93.34**
Cage Wash		**0.10**
Total Excretion		**93.49**

Elimination, summarized in the near-by table, was almost fully via feces and accounted for 93.34% of administered dose or 99.8% of the recovered excreted radioactive material. Urine, representing absorbed radioactive material, was 0.06% of the total amount administered.

Total recovery was 93.56% of administered radioactivity during the study period.

The presence of radiolabelled impurities in the test item dosing mixture is considered as the main source of the measured radioactivity in the absorption parameters and that they likely had a higher absorption rate than the test item. This is based on the difference in chemical purity of the ^{14}C-FAT 65080 of 99.59 % to 98.19 % purity in the dosing mixture. The reason for this difference can be accounted for by the small peaks preceeding (more polar) the ETH50 peak noted in chromatogram (RCC report, Figure 4, pg 34). The constituents of the 1.4% impurities were not identified or quantified given the small quantity of dosing mixture available and the relatively small amount of impurities. However, these impurities could have had higher absorption than that of the test item active ingredient thereby accounting for the measured radioactivity that could suggest falsely that absorption occurred. Regardless, the meaningful conclusion remains that the small particle sized ETH50 had only minimal absorption from the gastro-intestinal tract with subsequently very low bioavailability.

2.2.3 In vivo oral absorption, distribution, metabolism and elimination- rat

The in vivo absorption, distribution, metabolism, and elimination of micronized (d(0.5) <6.14 µm), radiolabeled FAT 65'080 was evaluated in male Han-Wistar rat according to OECD guideline number 417 (Toxicokinetics; April 1984) **(Ref. 4)**. The experiment was performed over 96-hours after a single oral gavage dose to achieve the following 5 objectives:

1) estimate for the oral route the rate and extent of intestinal absorption of the test item,
2) investigate the blood kinetics,
3) determine the pattern of tissue distribution of the test item,
4) determine the rates and routes of excretion of the test item, and
5) investigate the metabolite pattern in urine and feces extracts.

These results will facilitate interpretation and evaluation of results from our other non-clinical tests with oral gavage dosing and give an indication of bio-availability of the test item. Because dosing in this study was with a slightly larger but micronized, particle size of 6 µm ($d_{0.5}$) these results will provide a basis of comparison of toxicokinetics measured with 14C-ETH50 of 81- 92 nm particle size ($d_{0.5}$) as describe in the previous study summary above.

Non-radiolabeled FAT 65'080 (FAT 65'080/B; batch KOC00050/004E) had a purity of 98 %. The [^{14}C] labeled FAT 65'080 was synthesized by ABC Laboratories (Columbia, MO, USA) as [Triazine-U-^{14}C] with a radiochemical purity 99.59% and specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg), and labeled batch number 49336-1-61.

The dosing suspension was prepared as a mixture of non-labelled and labelled test material dissolved in chloroform to give [^{14}C]-labelled FAT 65'080 with a final specific radioactivity of 164 kBq/mg

(1.07 µCi/mg). After removing the residual solvent from the mixture, the remaining solid material was processed at the testing laboratory in a planet micro mill after addition of 3 ml carboxymethylcellulose (CMC) solution [0.5% CMC in 0.4% Tween 80, v/v] and zirconium milling balls. The resulting microsuspension was made to 20 ml with CMC solution and had a final concentration of 17.4 mg ETH50/ml suspension. This process used a different micro-mill than in the previous study and because it had lower efficiency could not prepare a particle size distribution that fully represented the marketed product. Determined by laser particle sizer, about 50% of the particles were below 6.14 µm in diameter and 10% less than 1.47 µm in diameter.

The test system was 13 male HanRcc:WIST (SPF): Wistar rats, outbred, SPF-quality of approximately 180 g body weight corresponding to about 7 weeks of age. An acclimatization period was at least five days to the laboratory environment and included one day to the metabolism cages; during acclimatization the animals were kept in groups of 1-3 rats under conventional hygienic conditions in Makrolon cages with standard soft wood bedding. The animals were kept in rooms maintained at standard conditions, i.e. a temperature of 22±3°C, a relative humidity of 40-70 % and a 12 hours light/dark cycle.

The rats were divided into one group of 4 rats for mass balance (Group 1) and a second group of 9 animals for determination of blood kinetics (Group 2); each group received similar doses.

Dosing of test item suspension was by gastric intubation at target volume of 0.5 ml per 100 g rat body weight and a nominal dose of 100 mg test item per kg body weight. The exact radioactivity administered was determined via weighing of the application device before (with administration solution) and after administration (empty). The amount of applied test item was calculated based on the concentration of [^{14}C] FAT 65080 in the administration solution. The radiochemical purity of the test item was checked by HPLC at the time of the application.

Urine and feces samples for each of four 24-hour periods were collected individually and separately per metabolism cage; urine was collected into containers on dry ice, feces at room temperature, and the daily collections for both substances stored frozen until analysis. Study termination at 96-hours after dosing was by CO_2 anaesthesia and exsanguinations, during which blood was collected from each animal and separately centrifuged to obtain plasma samples for analysis of radioactivity by LSC.

Cages were rinsed separately with water/ethanol (1:1 v/v) and the rinsates analyzed for collected radioactivity. From each animal, in addition to the blood and plasma collected, samples of each of the following were taken and weighed: liver, kidney, renal fat, and muscle; the remaining carcass was retained and processed for determination of radioactivity. Determination of radioactivity was made by LSC after the collected tissues, blood and organs were solubilized by addition of Solvable®, except for aliquots of urine, plasma, and cagewash, to which scintillation mixture was added and then the samples counted.

Blood kinetics were determined from serial blood samples of approximately 0.3 ml each withdrawn sublingual from 3 individual animals at the selected time points and collected into heparinized tubes. After taking aliquots of whole blood, it was separated into plasma and red blood cells by centrifugation at about 1500-2000 g for 10 min. Aliquots of plasma were taken for radiometry.

Three animals each were sacrificed by exsanguination after anesthesia with carbon dioxide at 24, 36 and 48 hours after administration. Terminal blood was collected into heparinized tubes and worked up analogously to the sublingual blood samples. The remaining specimens were stored frozen.

Metabolites in urine and feces were investigated after processing the samples separately for the 0-24 hours and 24-48-hours collection periods. For urine, aliquots representing 10 % of the total volume of each animal of Group 1 were pooled according to sampling time.

Group	Pooled Urine				
	Designation	Sampling Time	Volume	Radioactivity	Represents Percent of dose
1	U1 U2	0 - 24 h 24 - 48 h	4.0 ml 3.8 ml	475456 dpm 107550 dpm	0.53 0.12

Aliquots of U1 and U2 were used for HPLC metabolite profiling.
For feces, representative aliquots were about 10 % (0-24 h) and 20 % (24-48 h) of the total weight from each animal of Group 1 and were pooled according to sampling time.

Group	Pooled Feces				
	Designation	Sampling Time	Weight	Radioactivity	Represents Percent of dose
1	F1 F2	0 - 24 h 24 - 48 h	8.3 g 22.3 g	81.9 x 10^6 dpm 4.2 x 10^6 dpm	94.7 2.4

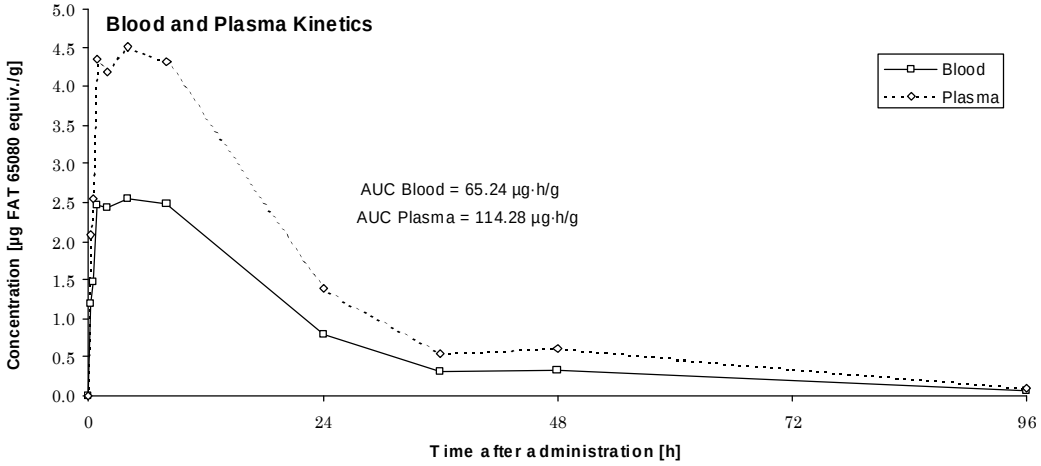
The feces pools were separately and exhaustively extracted at room temperature in a glass column using acetonitrile/water 80/20 (v/v) and acetonitrile => Extract 1, followed by tetrahydrofurane (THF) => Extract 2. The remaining solids were manually homogenized, and the amount of non-extractable radioactivity was determined by solubilization and subsequent liquid scintillation counting.

The volume of Extract 1 from each feces pool was reduced on a rotatory evaporator to about 2 to 10 ml, reconstituted by addition of methanol/tetrahydrofuran and tetrahydrofuran for Extract 1 and Extract 2, respectively. Aliquots of Extract 1 and Extract 2 were used for HPLC metabolite profiling.

Results. All 13 animals survived their specified study period and did not show signs of toxicity or adverse effects. Administered doses across both groups ranged from 98 to 108 mg ETH50 /kg body weight; the mean values were 103.7 mg/kg (3588 kBq/rat) for Group 1 and 100.7 mg/kg (3325 kBq/rat) for Group 2. The test item purity was 97.5 to 97.7% determined by HPLC analysis of the dosing mixture and remained stable during the 96-hours exposure period. Of note regarding the purity and impurity profile of the dosing material are the 3 small peaks preceding the ETH50 peak on HPLC 14C-chromatogram (RCC report, Figure 4, pg 37).

Absorption and Excretion. After oral administration the radioactivity was very poorly absorbed from the gastrointestinal tract into systemic circulation. The extent of absorption, calculated based on the urinary excretion, accounted for 0.73 % of the administered dose.

The maximum concentration level in blood and plasma was achieved 1 hour after administration, accounting for 2.463 and 4.359 µg FAT 65080 equivalents/g, respectively. This concentration level remained almost constant until 8 hours post dosing. Thereafter the concentration decreased with a half-life $_{(8\text{-}48\ h)}$ of about 13 hours. The AUC values $_{(0\text{-}96\ h)}$, being an index of bioavailability, were calculated to be 65.2 and 114.3 µg·h/g for blood and plasma, respectively. The radioactivity in blood was located predominately in plasma as indicated by the blood/plasma ratio of 1:1.8.



[RCC A89280]		Excretion [% of dose]
Urine	0 - 24 h	**0.53**
	24 - 48 h	**0.12**
	48 - 96 h	**0.06**
	Subtotal	**0.71**
Feces	0 - 24 h	**94.74**
	24 - 48 h	**2.44**
	24 - 48 h	**0.04**
	Subtotal	**97.22**
Cage Wash		**0.02**
Total Excretion		**97.95**

As summarized in the nearby table, 97.2% of the administered test item was excreted with the feces, which represented 99.2% of the recovered radioactivity within 48 hour after dosing. Only a very small amount of radioactivity was excreted with the urine, i.e. 0.71 % of the dose.

Distribution. Quantifiable amounts of the dosed radioactivity were recovered in each of the tissues sampled after 96 hours. The LOQ was 0.02 µg-equivalents per gram for each tissue, except for the remaining carcass with LOQ of 0.012 µg-equivalents per gram. Expressed as percent of dose applied, blood, liver, kidney and muscle were below 0.01%, while fat was 0.01 % and carcass 0.25% of dose. The next table summarizes the findings for the tissue distribution samples collected.

Residues 96 hours after administration					
[RCC A89280]	**µg-equivalents per g**			**percent of dose**	
	Mean	Std. dev.	LOQ	**Mean**	SD
Dose [mg/kg]	**103.7**	2.9	--		
Blood	**0.063**	0.008	0.022	**<0.01**	<0.01
Plasma	**0.099**	0.013	0.021	--	--
Liver	**0.109**	0.012	0.022	**<0.01**	<0.01
Kidneys	**0.088**	0.009	0.023	**<0.01**	<0.01
Fat	**1.712**	0.958	0.022	**0.01**	<0.01
Muscle	**0.038**	0.005	0.022	**<0.01**	<0.01
Carcass	**0.251**	0.051	0.012	**0.25**	0.06
			Total Residues	**0.27**	0.06

Metabolite pattern- Urine. The chromatography profiles revealed a simple metabolite pattern consisting of 7 metabolite fractions. Unchanged FAT 65080 was not found in urinary metabolite pattern as checked by co-chromataography with unlabeled test item. The low amount of each metabolite did not warrant their further identification. For the assessment of the urinary metabolite pattern it has to be considered that the total amount of urinary excretion (0.65 % of dose) was lower than the total amount of radiolabeled impurities (2.3 % of dose) that were administered with the test item. This indicates the metabolite pattern could represent radiolabeled impurities in the urine and not metabolites of ETH50, especially considering the pattern of radiochemical found in urine fractions more polar than ETH50 occurring at about the same relative retention time as those impurities seen in the dosing formulation.

Metabolite Pattern Urine

	% of dose		% of urine pool	
Pool	**U1**	**U2**	**U1**	**U2**
Sampling Time	0-24 h	24-48 h	0-24 h	24-48 h
Metabolite Fraction[+]				
U1	0.09	0.01	16.3	8.2
U2	0.08	0.01	15.5	11.3
U3	0.06	0.01	12.0	8.5
U4	0.06	0.01	10.8	8.2
U5	0.06	0.01	11.2	11.9
U6	0.03	0.02	5.9	14.2
U7	0.15	0.04	27.8	37.4
U8[#]	<0.01	<0.01	0.5	0.4
Total*	**0.53**	**0.12**	100.0	100.0

+ None of the fractions were identified further.
* Differences in last digit due to rounding.
# U8 corresponds to unchanged test item as shown by co-chromatography. A defined peak was not found at this retention time; therefore the given values represent the limit of quantification.

Metabolite pattern- Feces. For the investigation of the fecal metabolites the 0–24 and 24-48 hours feces were each pooled separately and extracted with acetonitrile/water 80/20 (v/v), acetonitrile, and THF. About 99 % (0-24 h) and 91% (24-48 h) of the feces radioactivity was extractable at room temperature (Extract 1 and Extract 2).

		Percent of pooled feces				**Percent of dose**		
Time interval	Designation	Total	**Extract 1**	**Extract 2**	Non-extractable	Total	**Extract 1**	**Extract 2**
0-24 h	F1	100.0	**1.6**	**98.2**	0.2	94.7	**1.5**	**93.0**
24-48 h	F2	100.0	**12.8**	**78.1**	9.1	2.4	**0.3**	**1.9**

The Extracts 1 and Extracts 2 were quantitatively analyzed by HPLC that revealed only unchanged FAT 65080, except for F1-1 in which 4 additional more polar metabolite fractions were found but in sum did not exceed 0.6 % of the dose. As for urine, the feces' chromatography pattern correlates well with the radiolabeled impurities in the dosing formulation, especially considering the pattern of radiochemical found in feces fractions more polar than ETH50 that occur at about the same relative retention time as those impurities seen in the dosing formulation (RCC report, Figure 11, pg 43).

Overall, it is concluded that the radiolabelled impurities present in the dosing mixture contributed significantly to the recovered radioactivity and the parent material showed very low absorption after oral administration. The larger particle size form of ETH50 (6 µm) was eliminated as unchanged

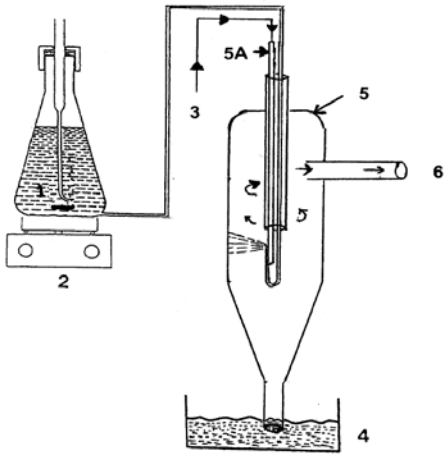
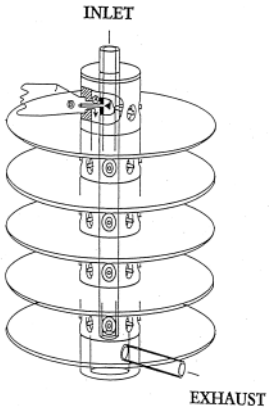
parent (>98% of dose) in feces and urine showed total radioactivity equivalent to about 0.6% of administered dose, which was about equal to those more polar fractions found in feces. While metabolism can not be excluded, it is more likely that the impurities (2.5% of dose) present in the dosing formulation are the source of radiochemical in these recovered fractions and suggest that they are more readily absorbed from the dosage formulation but do not accumulate.

2.3 Acute inhalation and respiratory response

The objective of this study was to assess the acute inhalation toxicity of FAT 65'080/F as a micronized small particle when administered to rats for a single 4-hour period (**Ref 5**). This study was designed to comply with OECD guideline for Testing of Chemicals No. 403, adopted on 12 May 1981, and with US EPA guideline OPPTS 870.1300, August 1998. In addition, the study examined lung inflammatory response markers via broncho-alveolar lavage fluid (BALF) sampling.

The test item, FAT 65'080/F, LOT04122FC7, contained 47.6% ETH50 from lot number 37874FC6 and lot number 37875FC6 prepared by micronization with excipients decyl glucoside, silicon defoaming agent, xanthan gum and butylene glycol; particle median diameter was 109 nm ($d_{0.5}$). A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of decyl glucoside, silicon defoamer, xantham gum, and butylene glycol, labelled FAT 65'080-placebo, was prepared. The test item and the placebo were prepared as aqueous dilutions at a ratio (w/w) of 20% FAT 65'080/F or 20% FAT 65'080-placebo plus 80% purified water, the target concentration as active ingredient of UV filter ETH50 was 10%.

Both the placebo and the test item aerosols were generated at ambient conditions using a cyclone glass atomiser that was operated at maximum throughput. Therefore, the aerosol concentrations

AEROSOL GENERATION SYSTEM	**NOSE-ONLY (FLOW-PAST) EXPOSURE APPARATUS**
	

1	Reservoir for aqueous test item dilution
2	Magnetic stirrer
3	Constant pressure airflow to atomiser
4	Dish collecting placebo or test item dilution not used for the aerosol
5	Glass atomiser (Cyclone 1000 mL, with stainless steel injector without baffle, with bottom hole and drainage pipe)
5A	Stainless steel injector without baffle
6	Outlet to the nose-only, flow-past exposure system

administered to the animals were considered to represent the highest technically achievable aerosol concentration levels suitable for acute inhalation toxicity testing in the rat. Animals were confined separately in restraint tubes that were positioned radially in the nose-only, flow-past exposure chamber as illustrated in the figure taken from the study report.

The concentration of the placebo and test item dilutions were determined gravimetrically and/or by chemical analysis, the particle size distribution determined gravimetrically, temperature, relative humidity and oxygen concentration were measured on test atmosphere samples collected directly from the aerosol delivery tube in the breathing zone of the animals.

The test system was rats of strain Wistar [HanRcc:WIST(SPF)] from RCC Ltd, Füllinsdorf, Switzerland. At the beginning of the experimental phase male animals were 9 weeks old and females were 10 weeks old and had a mean body weight of 244 g (range: 226 g to 262 g) for the males and 205 g (range: 187 g to 223 g) for the females.

A group of 15 male and 15 female rats was exposed by nose-only, flow-past inhalation to an aqueous dilution of FAT 65'080/F at a chemically determined mean aerosol concentration of 0.4976 mg/L air (s.d. ± 0.513 mg/L air, n = 4), equivalent to a gravimetrically determined mean concentration of 0.602 mg/L air (s.d. ± 0.059 mg/L air, n = 4). Two gravimetric measurements of particle size distribution during exposure indicated mass median aerodynamic diameters (MMADs) and geometric standard deviations (GSD) of 1.23 µm (GSD 2.13) and 1.27 µm (GSD 2.19).

Groups of 15 male and 15 female rats were exposed to an aqueous dilution of the control item, FAT 65'080 Placebo, at aerosol generation conditions similar to those used for the test item group. Two gravimetric measurements of particle size distribution during this exposure produced mass median aerodynamic diameters (MMADs) and geometric standard deviations (GSD) of 1.08 µm (GSD 2.25) and 1.10 µm (GSD 2.27).

	MMAD	Median droplet volume cm^3	No Particle a.i./L	Approx. Surface area (m^2) / L
Test item	1.2 µm	9.05E-19	1.15E+12	0.0275
Placebo	1.08 µm	6.60E-19	--	--

In each dose group, animals were subdivided in three satellite groups of five male and five female animals. The first satellite group was sacrificed about 14 hours post end of exposure for broncho-alveolar lavage fluid (BALF) and plasma sampling, the second group was assigned to interim pathology at approximately 24 hours post end of exposure (test day 2), and the third was assigned to pathology at 14 days post exposure (test day 15). All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. until interim sacrifice or over a 15-day observation period. Body weights were recorded before exposure on test day 1 in all animals and during the observation period on test days 4, 8 and 15 in all animals assigned to sacrifice on day 15.

The BALF examinations comprised total and differential cell counts and the determination of total protein, TNF-α and IL-6. In addition, total protein was determined in blood plasma from the animals assigned to BALF sampling. Pathology examinations comprised complete macroscopic pathology, the determination of lung weight and histopathology of the lungs and tracheobronchial lymph nodes on days 2 and 15.

Results.

The ranges of aerosol concentration, particle size, temperature, relative humidity, oxygen content, and airflow measured during both inhalation exposures were considered to be satisfactory for a study of this type.

Clinical signs attributable to treatment with the aqueous placebo dilution or test item dilution did not occur and no premature deaths occurred during the study. Three days after exposure (test day 4), group mean body weight was slightly lower in animals treated with the test item than in placebo control animals, attaining statistical significance only in female animals. This possibly test item-related effect was only transient and considered to be minor in degree, as no body weight loss was evident throughout the study.

In BALF collected at about 14 hours after end of exposure the total cell count (mainly macrophage and neutrophil numbers), TNFα and total protein were considerably higher in both sexes of test item-treated animals than in placebo control animals, while total protein levels in plasma did not distinguish the two groups. The results are summarized in the table below. The changes in BALF were consistent with the histopathology findings of granulocytic infiltration in alveolar wall and lumen, diffuse alveolar histiocytosis and alveolar lining cell activation seen in all test item-treated animals assigned to interim pathology at approximately 24 hours post end of exposure (test day 2).

By test day 15, these histopathology findings were no longer evident. Histopathology findings were not seen in placebo control animals. In addition, on test day 2, lung weights and lung to terminal body weight ratios were moderately higher in both sexes of test item-treated animals than in placebo control animals. By test day 15, the mean lung weight was only slightly higher in males of the test item group than in placebo group and was unaffected in females. Lung to body weight ratios did no longer differ significantly between test item and placebo control groups. Macroscopic pathology findings attributable to treatment with the test item were not evident.

BALF: Cell Count and Morphology; Cytokines and Protein Summary (mean ± std dev.)

Parameter	**Males** (N= 5)		**Females** (N= 5)	
	Placebo	**Test Item**	**Placebo**	**Test Item**
Total Cells (millions)	8.54 (3.03)	62.41**(16.29)	8.67 (0.79)	34.98**(16.68)
% of Total cells				
Macrophages	95.8 (1.4)	26.5 (1.3)[a]	92.5 (3.6)	36.7**(11.8)
Eosinophils	0.2 (0.2)	0.5 (0.7)[a]	0.1 (0.1)	0.3 (0.3)
Lymphocytes	0.8 (0.4)	0.5 (0.7)[a]	0.4 (0.4)	0.4 (0.4)
Neutrophils	0.9 (0.4)	71.0 (2.3)[a]	2.4 (2.4)	60.8**(12.1)
Other cells	0.3 (0.3)	0.9 (1.3)[a]	0.3 (0.2)	0.5 (0.5)
Epithelial cells	2.0 (0.7)	0.6 (0.8)[a]	4.3 (1.4)	1.2 (0.6)
TNFα (pg/ml)	<22.4	61 (17.73)	<22.4	46.2 (7.8)
IL6 (pg/ml)	61[b]	35; 226[c]	283; 35[c]	37; 72[c]
Total Protein (g/l)	68.1 (24.38)	317 (46)	153 (72)	243 (80.6)
Plasma Protein (g/l)	57.45 (2.33)	60 (1.61)	62 (1.2)	62 (2.04)

* / ** t-test based on pooled variance, significant at 5% or 1% level.
a. N = 2 animals; samples damaged. **b.** 4 rats <24 pg/ml **c.** 3 rats <24 pg/ml

Increases of total cell count, TNFα and total protein in BALF and of absolute and relative lung weight, and the histopathology findings of granulocytic infiltration, diffuse alveolar histiocytosis and alveolar lining cell activation seen in test item group on test day 2 were attributed to the treatment with the test item. Increase in neutrophil numbers in BALF on test day 2 was considered to be indicative of an inflammatory reaction.

Overall, the findings noted in test item-treated animals on test day 2 were considered to represent an acute clearance reaction to the lung burden of test item. This is further supported by the absence of these findings by test day 15 observations. This response is well documented for particle exposures to lung (Stone et al. 2007)[3] and the cellular response is not characteristic of immunologic response profiles reflected in the low numbers of lymphocytes at day 2 and their absence histologically at day 15 (Holt, P. et al. 2008)[4].

Such pulmonary inflammatory responses are not expected with use of ETH50 in sunscreens or other products from spray-on dispensers. Spray applicators are generally pump-type dispensers with some increase in marketed products using fine-spray aerosol type dispensers. In each of these applicators, the droplet sizes (aerosol) are designed to be at least 30 times larger than those used in this rat inhalation test. According to Durand et al. (2007)[5], sunscreen sprayable formulations should be dispensed with droplet size of more than 30 µm (MMAD) with not more than 1% of droplets of aerodynamic diameter at or below 10 µm.

For micronized ETH50 the inhalation LC50 is greater than the highest technically achievable aerosol concentration level of 4.976 mg/L air*, or greater than 0.4976 mg ETH50/L air, according to this study's results with MMAD of about 1.2 µm. A notable but reversible lung inflammatory response occurred but is considered a normal non-allergenic type response to particle exposures.*

2.4. Repeated Dose Toxicity

2.4.1 Oral gavage dosing toxicity and reproduction-developmental toxicity

The objective of this study was to evaluate the potential toxic effects of the test item, FAT 65'080/F as a micronized small particle, following daily oral gavage administration to male and female rats from before mating, through mating and, for the females, through gestation and until day 4 *post-partum* during lactation period (**Ref 6**). This study was designed to comply with OECD guideline for Testing of Chemicals No. 422, adopted on 22 March 1996, and with US EPA guideline OPPTS 870.3650, July 2000.

The test item, FAT 65'080/F LOT04122FC7, contained 47.6% ETH50 from lot number 37874FC6 and lot number 37875FC6, prepared by micronization with excipients decyl glucoside, silicon defoaming agent, xanthan gum and butylene glycol; particle median diameter was d(0.5) 109 nm; d(0.9) 175 nm.

Test Item Dose as mg a.i./kg b.wt	Total particles dosed (number)	No. Particles/kg body wt.	Surface area of particles dosed (m^2)
100	5.8E+13	2.31E+14	1.37
500	2.8E+14	1.15E+14	6.86
1000	5.8E+14	2.31E+15	13.7

Dosage formulations of the test item were prepared as suspensions in the vehicle (purified water) to achieve the concentrations of 42, 210 and 420 mg/mL and then homogenizing using a magnetic stirrer. At a dosing volume of 5 mL/kg/day, the target dose-levels as test item were 210, 1050 and 2100 mg/kg/day, respectively, or as active ingredient of UV filter ETH50: 100, 500 and

[3] Stone, V. et al. "Proinflammatory effects of particles on macrophages and epithelial cells." Ch 9 in Particle Toxicology, K. Donaldson & P. Borm editors. CRC Press, Boca Raton, FL 2007
[4] Holt et al. Regulation of immunological homeostasis in the respiratory tract. Nature Reviews- Immunology. Vol. 8: 142-152, 2008
[5] Durand et al. Influence of different parameters on droplet size and size distribution of sprayable sunscreen emulsions with high concentration of UV-filters. Int J Cosmetic Sci. vol 29: 461-471, 2007

1000 mg/kg body weight/day, respectively.

A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of decyl glucoside, silicon defoamer, xantham gum, and butylene glycol was prepared and labelled FAT 65'080/E-placebo. The placebo was diluted in distilled water and administered at a dose of 420 mg/mL, which was equivalent to that of the group receiving the highest dose of test item.

A control group of 10 males and 10 females were treated with distilled water during the study and served as comparison for placebo-treated group or test-item treated groups.

The test system was rats of strain Sprague-Dawley Crl CD® (SD) IGS BR, *Caesarian Obtained, Barrier Sustained-Virus Antibody Free* (COBS-VAF®) from Charles River Laboratories France, L'Arbresle, France. At the beginning of the treatment period the animals were at least 11 weeks old and had a mean body weight of 430 g (range: 399 g to 477 g) for the males and 244 g (range: 214 g to 283 g) for the females. The animals were sexually mature and the females were virgin. Each of the 5 study groups received 10 males and 10 females randomly assigned to treatment groups after a minimum of 7 to 13 days' acclimation period.

Daily dosing, at approximately the same time each day, was by gastric intubation as follows:
For the males:
- for 14 days before mating,
- during the 2 weeks mating and the 2 weeks post-mating periods until sacrifice (maximum of 6 weeks in total).

For the females:
- 14 days before mating,
- during the mating period (maximum of 14 days),
- during pregnancy and lactation, until day 4 *post-partum* inclusive, or until sacrifice for un-mated and non-pregnant females.

Day 1 corresponded to the first day of treatment period.

Clinical signs and mortality were checked daily. Body weight and food consumption were recorded weekly until mating and then at designated intervals throughout gestation and lactation. The animals were paired for mating and the dams were allowed to litter and rear their progeny until day 4 *post-partum* (p.p.). The total litter sizes and numbers of pups of each sex were recorded after birth, pup's clinical signs were recorded daily and pup body weights were recorded on days 1 and 4 *post-partum*. Calculation of reproductive success indices were made for parameters pre- and post-implantation loss, mating, fertility, and gestation. In parent animals from each group, hematology and blood biochemistry investigations were performed in five male and five female animals at terminal sacrifice. In addition, urinalysis was carried out in five males at terminal sacrifice.

The parent males were sacrificed 2 weeks after the end of the mating period. The body weight and principal organ weights (adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes, and thymus) were recorded, a complete macroscopic *post-mortem* examination was performed and selected organs/tissues were preserved. A microscopic examination was performed on selected organs for five males in control and high-dose groups, with particular attention paid to the male gonads for spermatogenesis staging and morphological structure.

The parent females were sacrificed on day 5 *post-partum* (or on day 25 *post-coitum* for females which did not deliver or 24 days after the end of the pairing period for unmated females) and a complete macroscopic examination was performed and selected organs/tissues were preserved. In females that were apparently non-pregnant, the presence of implantation scars on the uterus was checked using ammonium sulphide staining technique. A microscopic examination was performed on selected organs of five delivered females in control and high-dose groups.

The litters were sacrificed on day 5 *post-partum* and were carefully examined for gross external abnormalities and a macroscopic *post-mortem* examination was performed.

Results. The analytical chemistry portion of this study is now classified as not GLP compliant but remedial steps are in progress at the testing laboratory and the amended final report will be resubmitted in support of our dossier.

Throughout the dosing period, a satisfactory agreement was demonstrated between the test item concentration obtained in the dosage forms and the expected values.

Mortality included one male given the placebo found dead on day 28 without prior clinical signs. At necropsy the spleen was abnormally enlarged (approximately 13.5 cm long and 4 cm wide) and of irregular color. One control female found with her cannibalized litter was sacrificed on day 1 *post-partum*. Four mated females, one in control, two given the placebo, and one treated at 100 mg/kg/day were sacrificed on day 26 *post-coitum* after no delivery (These 4 were all confirmed not pregnant at necropsy.)

Test item treatment-related clinical signs did not occur during the study in males or females of any dosed group; control and placebo group animals were similarly not affected.

Differences were noted during the study between placebo control females and those given purified water: lower mean body weights and mean body weight gains associated with slightly low mean food consumption were noted in placebo control females through the pre-mating and gestation and lactation (minimally lower mean body weights only) periods. In all test item-treated groups, values recorded for these parameters were similar to controls given purified water. Clinical chemistry, hematology, and urinalysis results showed effects in some parameters of the test-item treated groups when compared to the placebo control group; however, when compared to the vehicle control group, these differences were not statistically or biologically significant and were considered as not attributable to the test item treatment.

Dose-level (mg/kg/day)	0 Purified water	0 Placebo (a)	100	500	1000 (b)
Number of pregnant females	9	7	9	10	9
Number of females surviving delivery	8	7	9	10	9
Mean duration of gestation (days)	21.1	21.1	21.3	21.3	21.2
Mean number of *corpora lutea*	19.0	16.6	19.0	17.0	17.0
Mean number of implantations	16.9	15.7	16.6	16.0	15.6
Mean number of pups delivered	16.1	14.1	15.9	15.3	13.4
Mean number of live pups on day 1 *p.p.*	15.8	14.0	15.4	15.1	13.3

The statistical analysis was performed first between placebo controls and purified water controls and then between placebo controls and dose-levels of 100, 500 and 1000 mg/kg/day.
(a): one female (N27410) excluded due to absence of evidence of mating.
(b): one female (N27440) excluded due to unknown male.

Reproductive data evaluation showed that neither the mating nor the fertility parameters were adversely affected by the test item treatment or administration of the placebo. However, in the placebo control females, a slightly low mean number of *corpora lutea*, and slightly low mean number of implantations, pups delivered and live pups on day 1 *p.p.* were obtained. The results for all groups are summarized in the table.

The observation of the pups after birth did not reveal any increased incidence of pups dying, adverse effects on the pup body weight gains or influence on the pups' sex ratio; furthermore, gross malformations were not found in any of the pups.

At the *post-mortem* examinations of the F0 generation parent animals, test item treatment-related macroscopic observations were not revealed. None of the differences in organ weights noted between the placebo and control groups or between the test item-treated and the placebo groups were considered to be of toxicological importance. The organ weights of test-item treated animals showed some statistically significant differences from the placebo control group among males and female. However, the differences could not be clearly assigned to the test item because of one or more confounding factors in the date including the affect of placebo on an organ's weight, the wide variation in individual animal's organ weights, or the test item group's results were within the weights' range of untreated control group. Importantly, in all cases, histological evidence for an adverse effect was not found.

Qualitative staging for testis did not indicate any abnormalities in the integrity of the various cell types present within the different stages of the spermatogenic cycle. The estrous stages were not affected by test item or placebo treatments, and microscopic abnormalities were not revealed in the evaluation of the ovarian follicles and *corpora lutea* or in the evaluation of the uterus.

Histological changes in all organs and tissues, except lung, observed and reported as minimal to slight severity were those commonly observed spontaneously in the untreated rat of this strain and age and none was considered to be attributable directly to the placebo or test item. Lung tissues showed cellular or repair changes in various numbers of females in each of the placebo control and test item treated groups that were normal, spontaneously occurring changes or were correlated with recorded incidences of dosing material reflux subsequent to intubation. The changes included mucoid material or bronchitis in 2 females of placebo group; slight focal interstial fibrosis in one low dose group female; minimal (2/5) foamy alveolar macrophage infiltration (vs 5/5 control & slight in 4/5 placebo) and minimal alveolitis in two of 5 females of the 500 mg/kg/day group; and large histiocytes in bronchiolar periphery at slight severity in 2/5 females. None of these changes in lung could be associated with a toxic effect of the test item but were secondary to accidental aspiration of the intubated material subsequent to treatment.

Overall, micronized ETH50 did not show adverse effects to the systemic toxicity endpoints, mating and reproduction parameters, offspring survival to 5 days' of age, or to grossly and microscopically evaluated organs and tissues.

The No Observable Effect Level is 500 mg ETH50/kg body weight/day and the No Observable Adverse Effect Level is 1000 mg ETH50/kg/day.

2.4.2 Subchronic 13-Week dermal toxicity study in rats

The objective of this study was to evaluate the potential toxicity of the test item, FAT 65'080 as a micronized (small) particle, following daily cutaneous application to rats for 13 weeks (**Ref 7**). On completion of the treatment period, designated animals were held for a 2-week treatment-free period in order to evaluate the reversibility of any findings. The study was designed to comply with the following guidelines: Note for guidance on repeated dose toxicity. Committee for proprietary medicinal products (CPMP/SWP/1042/99). European Agency for Evaluation of Medicinal Products, 27 July 2000 and OECD Guideline No. 411, 12th May 1981.

The test item, FAT 65'080/F LOT04122FC7, contained 47.6% ETH50 from lot number 37874FC6 and lot number 37875FC6, prepared by micronization with excipients decyl glucoside, silicon

defoaming agent, xanthan gum and butylene glycol; particle median diameter was d(0.5) 109 nm; d(0.9) 175 nm.

The test item was administered as a suspension in the vehicle. The vehicle was obtained by mixing Base ointment, hydrophilic (vehicle 1) and 0.5% carboxymethylcellulose aqueous solution in purified water prepared using purified water (vehicle 2) with the proportions of 80% of vehicle 1 and 20% of vehicle 2. The test item was mixed with the required quantity of vehicle in order to achieve the concentrations of 60, 200 and 400 mg/mL (expressed as active component FAT 65'080) or 126, 420 and 840 mg/mL (expressed as test item as received (FAT 65'080/F).

A placebo control mixture, containing all excipients in micronized ETH50 except the active ingredient, was provided by the sponsor and prepared into dose formulation by mixing the required quantity of vehicle and placebo in order to achieve the concentration of 840 mg of placebo/mL. Therefore, concentration of excipients in the placebo control dosage form was equivalent to the concentration of excipients administered in the highest dosage group.

The test system was Wistar Han rats designated Crl: WI (GLX/BRL/Han) IGS BRO; 107 males and 106 females were received at CIT on 22 February 2007 from Charles River Laboratories France, l'Arbresle, France. On the first day of treatment, the animals were approximately 8 weeks old and had a mean body weight of 244 g (range: 222 g to 274 g) for the males and 166 g (range: 144 g to 186 g) for the females.

The animals were acclimated to the study conditions for a period of 7 days before the beginning of the treatment period. The required number of animals (95 males and 95 females) was selected according to body weight and clinical condition during the acclimation period and allocated to the groups (by sex), using a computerized randomization procedure, so that the average body weight of each group was similar. Each animal was identified by an implanted microchip and a unique CIT identity number. The animals were housed individually in suspended wire-mesh cages with free access to food and water; room temperature, humidity, ventilation and light cycle were at standard settings for animal husbandry in a barriered rodent facility.

On the day before the beginning of the treatment period, an area corresponding to approximately 10% of body surface area (*i.e.* from 45 to 50 cm^2 in males and from 30 to 35 cm^2 in females according to their age/growth) was clipped free from hair, as close to the skin as possible on the dorsum of the animals, with an electric clipper. Before the first dosage form application the treatment area was examined and any animals showing skin abnormality and/or irritation were replaced from the spare animals ordered. The animals were clipped whenever necessary during the treatment period, but at least 4 hours before dosing and at least once a week. In addition, on the day before the first dosing, the animals of group 6 wore a protective plastic collar over a period of approximately 6 hours in order to habituate them wearing it.

Based on results of a 14-day dermal dose range finding study selection of dosages for the 13-week study were set at the dose-levels as active ingredient of 150, 500 and 1000 mg of FAT 65'080/kg/day; the study design is shown in the following table.

Group No.	Treatment		Number of animals	Dose-level[∇] (mg/kg/day)	Dose-level[#] (mg/kg/day)	Concentration of excipients (mg/mL)	Concentration of Active (mg/mL)
1	Control (untreated)	Principal	15 M 15 F	-	-	-	
2	Control (placebo/vehicle)[(a)]	Principal Satellite	15 M 15 F 3 M 3 F	0	0	440	0
3	Low-dose	Principal Satellite	10 M 10 F 3 M 3 F	150	315	66	60
4	Mid-dose	Principal Satellite	10 M 10 F 3 M 3 F	500	1050	220	200
5	High-dose I	Principal Satellite	15 M 15 F 3 M 3 F	1000	2100	440	400
6	High-dose II (with collar)	Principal Satellite	15 M 15 F 3 M 3 F	1000	2100	440	400

M: male; F: female.
(a): placebo was diluted in the vehicle at the concentration of 840 mg/mL (taking into account of the density).
∇: expressed as active component FAT 65'080.
#: expressed as test item as received (FAT 65'080/F).
-: untreated control group.

The cutaneous route was selected since it is the expected route of exposure in humans. The dosage form was applied to the dorsum uniformly using a plastic syringe fitted with a metal tube. For animals of groups 2 to 5, no dressing or protective plastic collar was used. At least 6 hours after each application the dose site was cleaned using purified water and dried with a cotton pad. Animals of group 6 wore a protective plastic collar for a period of at least 6 hours after each application in order to prevent ingestion of the test item. The collar was removed after each exposure period and the application site was cleaned using purified water and dried with a cotton pad. The quantity of dosage forms applied to each animal was adjusted according to the most recently recorded body weight. A constant dosage-volume of 2.5 mL/kg/day was used.

Mass dosed: mg a.i./kg body wt./ day	% a.i. applied	mg a.i. / cm^2 on dose site	Number a.i. Particles/ kg b.wt./d	Number a.i. particles/ cm^2	Specific surface area (m^2) dosed/cm^2
150	6	1.5	3.46E+14	3.46E+12	0.08
500	20	5	1.15E+15	1.15E+13	0.275
1000	40	10	2.31E+15	2.31E+13	0.549
1000	40	10	2.31E+15	2.31E+13	0.549
Estimated Human exposures (30 mg/kg bw)	10	0.2	6.93E+13	4.62E+11	0.011

Animals of group 1 (untreated control group) received neither treatment nor rinsing but clipping of the application site was conducted as for the other groups. The animals of group

2 (placebo/vehicle control group) received the placebo diluted in the vehicle. The dosage forms were stirred continuously throughout the dosing procedure.

A summary table is shown below for animal exposures in this study compared to usual human topical application rate of a theoretical sunscreen formulation containing 10% ETH50.

The dosage forms were administered daily for a period of at least 13 weeks (*i.e.* 91 to 92 days according to the necropsy schedule). At the end of the treatment period, the principal animals of each group were humanely sacrificed, except the first five surviving animals of each sex in groups 1, 2, 5 and 6, which were kept for a 2-week treatment-free period. The satellite animals were allocated to toxicokinetics investigations and those animals in groups 5 and 6 were kept for the 2-week treatment-free period.

The in-life phase of the study included the following parameters. The animals were checked daily for mortality and clinical signs and detailed clinical examinations were performed once a week. Body weight and food consumption were recorded once a week during the study. Neurotoxic evaluation using Functional Observation Battery (FOB) was carried out at the end of the treatment period on principal animals of the vehicle control and first high-dose groups (groups 2 and 5). Ophthalmological examinations were performed before the beginning of the treatment period on all principal animals and at the end of the treatment period on animals of the vehicle control and the first high-dose groups (groups 2 and 5). Hematology and blood biochemical investigations were performed on all principal animals at the end of the treatment and treatment-free periods. Urinalysis investigations were performed at the end of the treatment period.

Satellite animals (three males and three females in the control and test item treated groups (groups 2 to 6)) were allocated for the determination of the test item plasma levels and were sampled prior to dosing, on day 8 before the daily application and once during week 13.
On completion of the treatment period, designated animals were held for a 2-week treatment-free period. At the end of the treatment or treatment-free periods animals were killed and submitted to a full macroscopic *post-mortem* examination. Designated organs were weighed and selected tissue specimens were preserved. A microscopic examination was performed on selected tissues from animals of the vehicle control and the two high-dose groups (groups 2, 5 and 6) and on all macroscopic lesions in the low- and mid-dose groups.

Statistical analysis was performed as follows:
. by comparison of untreated group (group 1) and placebo/vehicle control group (group 2);
. by comparison of treated groups (groups 3 to 6) and vehicle control group (group 2);
. by comparison of high-dose groups (group 5 *versus* group 6).

Results. The analytical chemistry portion of this study is now classified as not GLP compliant but remedial steps are in progress at the testing laboratory and the amended final report will be resubmitted in support of our dossier.

Mortality
No test item-related deaths occurred.

Clinical signs
Scabs were noted at the application site in 6/15 males and 5/15 females from the high-dose I group given 1000 mg/kg/day (with no protective collar). This finding resolved by the end of the dosing period for almost all the animals except in 2/15 group 5 males. Clinical signs related to pain, such as abnormal vocalization and/or hyperactivity were observed, mainly from week 5, within the 30-minute

period after treatment and generally lasted for less than 30 minutes, in animals given the test item at the high dose-level of 1000 mg/kg/day, but mainly in the high-dose group with no protective collar.

Detailed clinical examination and Functional Observation Battery
The functional test battery showed no test item treatment-related changes in any neurotoxicological parameter. Motor activity was not affected by the test item treatment.

Body weight
When compared to untreated animals, a slightly lower mean body weight gain was observed in placebo control males (-15% when compared to untreated controls) during the treatment period. This effect was attributed to the placebo treatment.
In all males given 1000 mg/kg/day (groups 5 and 6) statistically significantly ($p<0.05$) lower mean body weight gain was recorded, principally from week 2 and during the whole study period. Mean body weight and mean body weight gains for females of both high dose groups (groups 5 and 6) were similar during the full study period.
The group 5 and 6 males given 1000 mg/kg/day gained 13 and 9 grams more weight respectively than placebo controls during the treatment-free period, thereby showing full reversal of their body weight trends observed during the treatment period.

Food consumption
Mean food consumption was not affected by the placebo or test item treatment in any period.

Ophthalmology
There were no ophthalmological findings at the end of the treatment period.

Hematology and blood biochemistry
Differences of toxicological significance did not occur between control and test item treated animals in any hematological or blood biochemical parameters. The lower glucose and triglycerides concentrations observed in test item high dose group males (groups 5 and 6) at the end of the treatment period were considered to be related to the treatment procedure. The change in females triglyceride results were not of toxicological significance.
Urinalysis
There were no disturbances in any urinalysis parameters at the end of the treatment period.

Plasma levels of the test item
All group 3 to 6 animals showed quantifiable amounts of the active ingredient of the test item during the study period, suggesting a systemic exposure occurred although no time (duration of exposure) or dose-related patterns were demonstrated. The analytic level of quantification was 0.8 ng/ml. Only parent ETH50 was found in the analyzed samples. The results are summarized in the following table.

Plasma ETH50 Concentrations (ng/ml) in 13-Week Dermal Rat Study										
	Male					Female				
Group Number	2	3	4	5	6	2	3	4	5	6
Dosage (mg/kg/day)	0	150	500	1000 High-dose I	1000 High-dose II	0	150	500	1000 High-dose I	1000 High-dose II
Day 8	0	0.79	1.36	4.17	1.21	0	0.36	5.62	3.04	1.39
Week 13	0	2.39	3.48	2.36	2.47	0	2.05	9.82	2.10	11.81
Week 15 (after reversibility)	*n.a*	*n.a*	*n.a*	1.98	0.39[▽]	*n.a*	*n.a*	*n.a*	0.39	<0.8[#]

[▽]: mean concentration with exclusion of the N26873 value (16.8 ng/mL), considered to be aberrant
n.a: not applicable.
[#]: missing values for animals N27054 and N27055

Organ weight
Organ weight changes were recorded in the thymus of group 5 males, and the adrenal glands of group 5 females. Although no histopathologic changes were observed in these tissues in rats of this dose group, the changes were considered as non-specific indicators of a stress-related response.

Macroscopic *post-mortem* examination
Except for scabs noted in the treated application site of 2 group 5 males, no test item related macroscopic findings were observed.

Microscopic examination
Epidermal hyperplasia and associated hyperkeratosis were noted in rats from groups 2, 5 and 6, which received similar doses of excipients either directly or mixed with test item. These findings were considered likely to reflect mild non-specific irritant-effects related to the mechanical preparation of the application site and repeated treatments with equal excipient concentrations to the application site. Minimal increased lymphocytolysis was recorded in a few rats of groups 5 and 6 (*i.e.* mainly females of group 5). This finding may reflect a minimal non-specific stress-related response related to the treatment procedure.

At the dose-levels of 150 and 500 mg/kg/day, no signs of local or systemic toxicity were noted.

Conclusions. Under the experimental conditions of this study, the **No Observed Adverse Effect Level (NOAEL) of ETH50 (FAT 65'080) is 1000 mg/kg/day as active ingredient** given by cutaneous application to rats of the FAT 65'080/F test item form during 13 weeks.

Additional Comment (July 2010): Examination of the frequency and occurrence of clinical signs for individual animals including abnormal vocalization of short duration, hyperactivity, dermal scabs, indicated they occurred only the 2 high dose groups receiving 1000 mg/kg/d, with and without restriction collar. Considered together with the decreased body weight gains that fully reversed during the treatment-free period and the observed lymphocytolysis in high dose groups animals, and the absence of remarkable findings in the Functional Observational Battery in any of the animals tested, we conclude that the study results demonstrated a "maximally tolerated dermal dose" was achieved with 1000 mg/kg/day. Further, the absence of other systemic adverse effects and any microscopic adverse changes in tissues and organs further support the NOAEL of 1000 mg/kg/day in this study.

2.5 Mutagenicity/Genotoxicity

2.5.1 Bone marrow micronucleus test, mouse

The objective of this study was to evaluate the potential of the test item FAT 65'080/E, containing micronized small particle sizes, to induce damage to the chromosomes or the mitotic apparatus in bone marrow cells of mice **(Ref. 8)**. Tested in parallel was the reference item FAT 65'080/B, which contained the active ingredient in non-micronized particle sizes.

This test was performed according to the method described by Schmid (1975)[6] and modified by Salamone etal. (1980).[7] This study's design was in accordance with OECD guideline No. 474, adopted on 21 July 1997 and EU Commission Directive No. 2000/32/EC, B12, 8 June 2000.

The test item, FAT 65'080/E contained 49.5% ETH50 of batch number KRG328-2 prepared by micronizing ETH50 LOT11106CL4AA; a subsequent analysis of the test item sample indicated a purity of 50.6% a.i., and a particle median diameter of 81 nm ($d_{0.5}$).
Dosage formulation of the test item was by suspending it in the vehicle (purified water) to achieve the concentrations of 50, 100 and 200 mg/mL and then homogenizing using a magnetic stirrer. At a dosing volume of 10 mL/kg, the target dose-levels as test item were 500, 1000 and 2000 mg/kg/day, respectively, or as active ingredient of UV filter ETH50: 250, 500 and 1000 mg/kg/day, respectively.

A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of decyl glucoside, xantham gum, and butylene glycol, labelled FAT 65'080/E-placebo was administered at a dose equivalent to that of the group receiving the highest dose of test item. The placebo was dissolved in the vehicle to achieve the concentration of 100 mg/mL and then homogenized using a magnetic stirrer. Using a treatment volume of 10 mL/kg, the target dose-level was 1000 mg/kg/day.

The Reference material, FAT 65'080/B was from ETH50 batch No. KOC00050/004.E with a purity of 98%; the median particle size, $d_{0.5}$, was 15.4 µm. This substance was not micronized and was used as a comparison to assess if any observed effects may be attributable to particle size differences. Dosage form preparation was by suspension in the vehicle in order to achieve the concentration of 200 mg/mL and then homogenization using a magnetic stirrer. Using a treatment volume of 10 mL/kg, the target dose-level was 2000 mg/kg/day as the active ingredient ETH50. Equivalent dosing of micronized a.i. as particle number and specific surface area are shown in the nearby table.

Test Item Dose as mg a.i./kg b.wt	Total particles dosed (number)	No. Particles/kg body wt.	Surface area of particles dosed (m^2)
250	1.73E+13	5.77E+14	0.41
500	3.46E+13	1.15E+15	0.82
1000	6.93E+13	2.31E+15	1.65

The positive control was Cyclophosphamide (CAS No. 50-18-0, CPA, Sigma, Saint-Quentin-Fallavier, France), batch No. 084K1328 dissolved in distilled water at a concentration of 5 mg/mL. The preparation was stored at -20°C and thawed immediately before use.

The study was conducted in Swiss Ico: OF1 (IOPS Caw) mice. A preliminary toxicity test was

[6] Schmid W (1975). The micronucleus test. Mutation Research, 31, 9-15.
[7] Salamone M, Heddle J, Stuart E and Katz M (1980). Toward an improved micronucleus test. Studies on 3 model agents, mitomycin C, CPA and dimethylbenzanthracene. Mutation Research, 74, 347-356.

performed to define the dose-levels to be used for the cytogenetic study. In the main study, one group of five males and five females received the positive control test item (Cyclophosphamide) once by oral route at the dose-level of 50 mg/kg. An additional three groups of five males and five females mice were given intraperitoneal administrations of test item, placebo, or reference material at dosages cited above. The high dose test item group and the reference material group retained satellite groups of 3 male and 3 female mice for blood sampling after dosing and determination of test item in plasma. Blood samples for these determinations were taken from 3 mice per sex at 1 hour (satellite animals) and 24 hours (at terminal sacrifice on 3 out of 8 animals of each sex) after the second treatment.

At the time of sacrifice, all the animals were killed by CO_2 inhalation in excess. The femurs of the animals were removed and the bone marrow was flushed out using fetal calf serum. After centrifugation, the supernatant was removed and the cells in the sediment were resuspended by shaking. A drop of this cell suspension was placed and spread on a slide. The slides were air-dried and stained with Giemsa. The slides were coded so that the scorer is unaware of the treatment group of the slide under evaluation ("blind" scoring).

For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes; the polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

From the preliminary toxicity testing with the test item, the top dose-level for the cytogenetic test was selected according to the criteria specified in the international guidelines. Because no toxic effects occurred the top dose-level was 2000 mg/kg/day; the two other selected dose-levels were 1000 and 500 mg/kg/day.

No clinical signs and no mortality occurred in the animals of both sexes either given placebo mix (1000 mg/kg/day) or test item at 500 and 1000 mg/kg/day. At 2000 mg/kg/day, the test item treated group showed hypoactivity and piloerection. The reference item treated group at the same dose-level showed hypoactivity, piloerection and soft feces.

The test item treated males and females had mean values of MPE and a PE/NE ratio that were equivalent to those of the vehicle control group.

For either placebo or reference item treated groups, the mean values of MPE were equivalent to those of the vehicle control group. The PE/NE ratio of females treated with the reference item was significantly lower than that of the vehicle control group.

i.p. Dose 2000 mg/kg	ng a.i/ml plasma	
Micronized a.i.	**Male**	**Female**
1 hour after dose	7560	6720
24-Hr after dose	6053	4020
Not Micronized		
1-hour after dose	3	2
24-Hr after dose	4	4

The mean values of MPE as well as the PE/NE ratio for the vehicle and positive controls were consistent with our historical data. Cyclophosphamide induced a significant increase in the frequency of MPE, indicating the sensitivity of the test system under our experimental conditions. The study was therefore considered valid.

Intraperitoneal dosing with micronized and not micronized test item resulted in measurable plasma concentrations of the active ingredient ETH50 at 1- and 24-hours after dosing as shown in the nearby table. Micronized ETH50 was associated with plasma concentrations about 1000 times higher than from not micronized ETH50, but in both cases the bone marrow has been exposed to the test item.

Under our experimental conditions, the test item FAT 65'080 did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two intraperitoneal administrations, at a 24-hour interval, at the dose-levels of 500, 1000 and 2000 mg/kg/day.

2.5.2 Unscheduled DNA synthesis in hepatocytes, rat

An *in vivo* / *in vitro* unscheduled DNA synthesis (UDS) assay was performed according to the OECD guideline 486 **(Ref. 9)**. The objective of this study was to evaluate the potential of the test item FAT 65'080/E, containing micronized small particle sizes, to induce DNA damage or lead to increased repair synthesis of the genome. Tested in parallel was the reference item FAT 65'080/B, which contained the active ingredient in large particle sizes.

The test item, FAT 65'080/E, contained 49.5% ETH50 of batch number KRG328-2 prepared by micronizing ETH50 LOT11106CL4AA in decyl glucoside, xanthan gum, and butylene glycol; a subsequent analysis of the test item sample indicated a purity of 50.6% a.i., and a particle median diameter of 81 nm ($d_{0.5}$).

The Reference material, FAT 65'080/B was from ETH50 batch No. KOC00050/004.E with a purity of 98%; the median particle size, $d_{0.5}$, was 15.4 µm. This substance was not micronized and was used as a comparison to assess if any observed effects may be attributable to particle size differences.

A placebo group was used to address effects from the excipients; accordingly, a mixture of decyl glucoside, xantham gum, and butylene glycol, labelled as FAT 65'080/E-placebo was administered at a dose equivalent to that of the group receiving the highest dose of test item.

Dosage forms were prepared by dilution with distilled water except the reference item was suspended in CMC (0.5% in distilled water). The test system was male Fischer rat from Charles River France, 5-6 weeks of age at time of expression time sampling. Based on a preliminary toxicity test, the selected doses of the test item were 1000 and 2000 mg/kg body weight, 2000 mg/kg for the reference item and 1000 mg/kg body weight for the placebo. Dosing was once by oral gavage at 10 ml/kg body weight and each group consisted of 3 males. The particle equivalent dosages from the micronized ETH50 are shown in the above table. Positive control substances were dimethylhydrazine at 10 mg/kg for the 2-4 hour expression time and 2-acetamidofluorene at 25 mg/kg for the 12-16 hour expression time.

Test Item Dose as mg a.i./kg b.wt	Total particles dosed (number)	No. Particles/kg body wt.	Surface area of particles dosed (m^2)
500	2.31E+14	1.15E+15	5.49
1000	4.62E+14	2.31E+15	10.98

Blood samples were collected after sacrifice for determination of test item concentrations.

Hepatocytes were collected after liver perfusion and removed to culture well-plates for radiolabelling; 12 culture wells per animal were prepared as slides. Autoradiography was conducted with 6 slides, 6 were held as backup if needed, and where possible 50 cells per slide from 3 slides per animal were evaluated for grain counting classified as nuclear (NC) or cytoplasmic (CC) grain counts, and calculation of net nuclear grains per cell (NNG = NC-CC).

As shown in the nearby tables, the test item, placebo and reference item did not cause increased net nuclear grain counts, did not increase the frequency of cells in repair, or induce cellular proliferation as seen in frequency of cells in S-phase. The viability of the hepatocytes was not affected due to the in vivo treatment either with the test article, placebo or reference item. The positive control items each gave responses indicating a robust and responsive test system.

Results of UDS Assay with ETH50 (FAT 65'080)

Assay I: Expression time 12-16 hours

12-16 hours expression time	DOSES in mg/kg	Net Nuclear Grain Count NNG	Net Nuclear Grain Count of cells in repair NNG≥5	% cells in repair NNG≥5	% cells in S-Phase
		Mean	Mean	Mean	Mean
Vehicle control	0	-1.65	5.65	1.49	0.14
FAT 65'080	2000	-1.77	5.63	1.81	0.09
	1000	-1.83	6.19	1.78	0.14
FAT 65'080/E - Placebo	1000	-1.69	5.82	2.17	0.00
FAT 65'080/B	2000	-1.78	5.59	1.55	0.16

Assay II:Expression time 2-4 hours

2-4 hours expression time	DOSES in mg/kg	Net Nuclear Grain Count NNG	Net Nuclear Grain Count of cells in repair NNG≥5	% cells in repair NNG≥5	% cells in S-Phase
		Mean	Mean	Mean	Mean
Vehicle control	0	-1.37	5.53	1.38	0.07
FAT 65'080	2000	-1.09	5.60	1.59	0.08
	1000	-1.28	6.08	1.69	0.09
FAT 65'080/E - Placebo	1000	-1.31	6.53	1.47	0.25
FAT 65'080/B	2000	-0.92	6.12	1.98	0.10

Plasma samples' analysis showed ETH50 was not detectable above LOQ of 0.6 ng/ml plasma; however, an isomer of ETH50 with same ion fragment and molecular mass was seen at Retention Time 4.20" and showed measurable concentrations after dosing with either micronized (FAT 65'080/E) or not micronized (FAT 65'080/B) ETH50 but not from placebo. The concentrations of RT4.20" isomer were higher at 2 hours than at 12 hours and the not micronized form was higher than micronized test item. It is our opinion that this isomer was more readily absorbed than the ETH50 and that this opinion is consistent with those results seen in the ADME study summarized above. Subsequent analytical work indicated that this isomer is a by-product known to be present at very low concentrations (<0.03% w/w) in each batch of test item used for all of the non-clinical testing conducted with ETH50. We consider these plasma results further demonstration of the low bio-availability of ETH50 parent molecule and that the test results for this UDS Assay are interpretable as ETH50, including by-products or impurities, being not mutagenic or genotoxic.

Accordingly, genotoxic effects (DNA damage) and cellular proliferative effects did not occur after in vivo dosing with ETH50 micronized, the placebo, or with ETH50 as large sized material. The small-sized particle did not show a different genotoxic response profile from the not micronized ETH50 and both are considered to be not genotoxic in this UDS assay.

2.6. Phototoxicity, photoirritation and photosesntization

2.6.1. 13-Week Phototoxicity study in hairless mice

The purpose of this study was to provide information for use in evaluating the potential for toxic or possible interactive effects associated with repeated daily administration of FAT65'080/E, with or without simulated sunlight, for a period of 13 weeks. (**Ref 10)**The study supports the topical safety of micronized ETH50 in the presence and absence of UV irradiation when applied in a lotion vehicle to hairless mice at doses at and up to 4 times higher than anticipated human topical dose rates from sunscreen application.

Eighty four male and eighty four female albino hairless Crl:SKH1-hr mice were randomized to fourteen groups, six mice per sex per group, as outlined in the below study design table.

Group	Description	Formulation Concentration (mg/g)	UVR Exposure per Week (RBU)	Administration Volume (mcl/mouse)
1	No Administration	Not Applicable	None	None
2	Vehicle[a]	0	None	100
3	FAT65'080/E	25	None	100
4	FAT65'080/E	50	None	100
5	FAT65'080/E	100	None	100
6	FAT65'080/E	200	None	100
7	No Administration	Not Applicable	600	None
8	Vehicle[a]	0	600	100
9	No Administration	Not Applicable	1200	None
10	Vehicle[a]	0	1200	100
11	FAT65'080/E	25	1200	100
12	FAT65'080/E	50	1200	100
13	FAT65'080/E	100	1200	100
14	FAT65'080/E	200	1200	100

a. Aloe Vera Lotion
Abbreviations: UVR:Ultraviolet Radiation ; RBU: Robertson-Berger Units

The ultraviolet radiation (UVR) source used was a 6.5 kilowatt xenon long arc, water cooled burner vertically-suspended within an octagonal metal frame holding one optical filter on each side. Each filter (15 cm by 15 cm, 1 mm thick; Schott WG 320 doped glass) was held approximately 20 cm from the burner. The racks holding the mouse cages were located approximately 2.25 meters from the UVR source during exposure. Each rack of cages was irradiated through one filter; all racks of cages are irradiated simultaneously from one xenon arc. Each rack of animal cages was monitored by a customized detector that records both intensity and UVR dosage in Robertson-Berger Units (RBU). The RBU is a measure of skin response to UVR; 400 RBU approximates one minimal erythema dose (MED) on previously untanned human skin.

The test item was ETH50 (LOT11106CL4AA) micronized in decyl glucoside, xanthan gum, and butylene glycoland labelled as FAT 65'080/E (KRG328-2); after this study was completed the test item sample was again evaluated for purity (50.6% active ingredient) and particle size distribution characterized as d(0.5)=81 nm; d(0.9)=157 nm. Dosages are expressed as active ingredient (a.i.) and summarized in the following table.

mg a.i./g dose formulation	% a.i.	mg a.i. / cm^2 on dose site	Approx. mg a.i./kg body wt./ day	Approx. No. a.i. particles/ cm^2	Specific surface area $(m^2)/cm^2$
25	2.5	0.1	80	2.3E11	0.005
50	5.0	0.2	160	4.6E11	0.010
100	10	0.4	325	9.2E11	0.020
200	20	0.8	650	18.4E11	0.040
Approximate Human Exposures	10	0.2	60	4.6E11	0.011

The test article formulations and/or vehicle were administered at a dosage volume of 100 microliter/mouse and the appropriate mice were irradiated once daily, 5 days per week, for 13 weeks as outlined in the Study Design Tables. Formulations were administered to the back and sides (approximately 25 cm^2) of appropriate mice before daily UVR exposure on Monday, Wednesday and Friday and after UVR exposure on Tuesday and Thursday. On Monday, Wednesday and Friday, UVR exposure began no later than 15 minutes after the completion of formulation administration for each group. On Tuesday and Thursday, the duration of time between the completion of UVR exposure and the start of formulation administration for each group of mice was no longer than 15 minutes.

Clinical observations and skin observations were recorded at least once weekly. Erythema, edema, flaking, or any other abnormal findings were recorded when observed and the intensity of these observations is described in terms of an internationally accepted standard, which is a modified Draize system. Body weights were recorded at least weekly throughout the study. Skinfold thickness for each mouse was measured using a thickness gauge before administration of any formulation (0 week) and in weeks 4, 8 and 13.

After completion of the 13-week dosage period, the mice were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Gross lesions found were retained in neutral buffered 10% formalin for possible histopathological examination. Samples of skin from the site of formulation administration for each mouse (or the equivalent anatomical location for untreated mice) were processed for possible histopathological examination. These histopathological examinations were not performed; the remainder of the carcass was discarded without evaluation.

Results. Test article residue was observed in all groups of male mice, without or with UVR exposure administered the FAT65'080/E formulations, as compared with the group of mice not administered any formulation. This finding is a characteristic of the test article and not considered adverse.

All male and female mice survived to scheduled sacrifice. The only necropsy finding in male mice was a tan mass in one mouse not administered any formulation and exposed to 1200 RBU/week of UVR and one male mouse administered 50 mg/g FAT 65'080/E and exposed to 1200 RBU/week of UVR. These findings were considered not related to the UVR exposure or FAT 65'080/E administration. All other tissues appeared normal.

Over the course of the study, no significant differences in group mean body weight occurred across the groups of male or female mice administered the vehicle formulation or the FAT 65'080/E formulations, without or with UVR exposure, as compared with groups not administered any formulation or administered vehicle formulation, without or with UVR exposure. Instances of significant changes (increases and reductions) in group mean body weight occurred, as compared with groups not administered any formulation or administered vehicle formulation, without or with UVR exposure. These changes in group mean body weights were not considered related to formulation

administration and/or UVR exposure or biologically important because: 1) the occurrences were not administration volume-dependent; 2), the changes were both increases and reductions; and/or 3) the occurrences were intermittent.

For male and female mice throughout the study, there were scattered instances of significant changes in mean skinfold thickness changes in both male and female mice administered the formulations, without or with UVR exposure, as compared with groups not administered any formulation without or with UVR exposure. These significant changes were not considered adverse because of the lack of dose and time-dependence and lack of a pattern of response that would indicate a clear association of the test item with this clinical endpoint.

The topical administration of micronized ETH50 with and without UV radiation did not adversely affect edema formation, wrinkling, or skin fold thickness in any group of males or females; these endpoints showed an increase in male and female mice treated with the vehicle alone or with UVR alone. This indicates ETH50 in micronized formulation does not generate alternative toxic forms or increase the adverse effects of UV irradiation and leads to a decreased incidence of markers of UV radiation damage indicative of an efficacious protective effect.

3. TOXICOLOGICAL EVALUATION:

DISCUSSION

Overview of results for the Additional Studies performed with ETH50 and used for the calculation of the margin of safety:

Study	Batch & Purity Particle size	Results
Non-clinical		
Acute		
Acute Oral Toxicity in Rats	KRG328-2; 49.5% ETH50 d(0.5): 81 nm	LD_{50} > 2000 mg/kg
Acute Inhalation Toxicity in Rats	Lot04122FC7; 47.6% ETH50; $d_{0.5}$:109 nm. Aerosol: MMAD 1.2 µm	LC50 > 0.4976 mg/L air (highest achievable ETH50 concentration); reversible inflammatory response to particles.
Repeated dose toxicity		
Oral gavage dosing toxicity and reproduction-developmental toxicity	Lot04122FC7; 47.6% ETH50; d(0.5): 109 nm	NOEL = 500 mg/kg/d NOAEL = 1000 mg/kg/d
90-Day Dermal Toxicity Study in the Rat	Lot04122FC7; 47.6% ETH50; d(0.5): 109 nm	NOAEL = 1000 mg/kg/d
Genotoxicity		
In vivo, Bone marrow micronucleus test, i.p. dose to mice	KRG328-2; 49.5% ETH50 d(0.5): 81 nm	Negative
In vivo / *in vitro* unscheduled DNA synthesis (UDS) assay (oral gavage, rat)	KRG328-2; 49.5% ETH50 d(0.5): 81 nm	Negative
Phototoxicity		
13-Week Phototoxicity study in hairless mice; topical dosing	KRG328-2; 49.5% ETH50 (end of the study re-analysis: 50.6% ETH50). d(0.5): 81 nm	No increase of adverse effects related to ETH50 with or without UV irradiation.

<table>
<tr><th>Study</th><th>Batch & Purity
Particle Size</th><th>Results</th></tr>
<tr><td>Absorption</td><td></td><td></td></tr>
<tr><td>In vitro percutaneous penetration, rat & human skin</td><td>[Triazine-U-14C] radiochemical purity >99%; specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg); batch no. 49336-1-61.
d(0.5): 92 nm to 81 nm

Not radio-labelled from KOC00050/004.E, purity of 98%
d(0.5): 15.4 µm</td><td><table><tr><th></th><th>Rat</th><th>Human</th></tr><tr><td><u>Flux</u> (0-24h) (µg-eq/cm^2/h)</td><td>0.035</td><td>0.026</td></tr><tr><td><u>Absorbed</u>
As % dose:
As µg/cm^2</td><td>
1.38
27.2</td><td>
0.06
1.2</td></tr></table></td></tr>
<tr><td>In vivo oral absorption rats:
Single oral gavage</td><td>[Triazine-U-14C] radiochemical purity >99%; specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg); batch no. 49336-1-61.
d(0.5): 92 nm to 81 nm

Not radio-labelled from KOC00050/004.E, purity 98%
d(0.5): 15.4 µm (not micronized)</td><td>Blood AUC: 17.9 µg-hr/g
Tissue conc: all <LOQ (~0.09µg/g) at 96-hours
Elimination as % of dose:
Urine 0.06%
Feces: 93.3%</td></tr>
<tr><td>In vivo oral absorption, distribution, metabolism and elimination- rat, single oral gavage</td><td>[Triazine-U-^{14}C] radiochemical purity ≥99%; specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg); batch no. 49336-1-61.
d(0.5): 6 µm

Not radio-labelled from KOC00050/004.E, purity 98%
d(0.5): 15.4 µm</td><td>Blood AUC: 65.2 µg-hr/g
Plasma AUC: 114.2 µg-h/g
Tissue conc. All <LOQ (~0.02 µg/g) except fat 0.01% and carcass 0.25% of dose at 96-hr
Elimination as % of dose:
Urine 0.65 %
Feces: 99% as parent</td></tr>
</table>

<u>Particle size effects</u>. The additional completed non-clinical studies with micronized ETH50 used a worst case small particle sized ($d_{0.5}$ 80 nm) sample and in different studies one approximating the product specification ($d_{0.5}$ 110 nm); in these additional tests, increased or different toxicity did not occur. That is, the small particle size of ETH50 did not show an increased level of toxicity in general toxicity and reproductive/developmental toxicity endpoints compared to not micronized (15µm $d_{0.5}$) ETH50 given for 13 weeks by oral gavage to rats at similar Mass/body weight dosages. Genotoxicity or mutagenicity was not apparent in the in vivo studies using both not micronized and micronized ETH50. Subchronic topical dosing to rats at maximally tolerated dosage was without marked signs of systemic toxicity attributable to the test item directly; a subchronic topical dosing study in hairless mice together with or without UV irradiation showed decreased (protective) effects of UV irradiation compared to placebo or UV-irradiation control groups. In the several studies, the particle dosages <u>approximated 10^{14} in total number or per body weight; topical dosages were about 10^{13} particles per cm^2</u> of skin area dosed.

In vitro percutaneous penetration studies of rat and human skin showed rat skin as more permeable than human skin and in both species, micronized ETH50 with an absorption profile about 10-fold lower than that of the larger micronized particles (6 µm) of ETH50. This comparison is summarized in the following Table 1 below.

Influence of particle size was also apparent from direct comparison in the mouse in vivo micronucleus assay wherein the intraperitoneal dosing of the micronized ETH50 resulted in plasma concentrations of a.i. about 1000 times higher than resulted from dosing with the not micronized form. A similar plasma concentration difference did not occur in rats given the two particle sizes via oral gavage. In the i.p. dosing study, it can be surmised that the micronized particles were more readily taken up by macrophages or lymphocytes than the larger sized particles of ETH50 resulting in the phagocytized particles relayed to lymphatic and systemic circulations more rapidly than a.i. was solubilized and absorbed from the not micronized particles of ETH50. We do not have evidence that whole particles of ETH50 are present in the analyzed tissues or fractions of these various studies; furthermore, we do not find it meaningful to determine if this is the case because toxicity or adverse effects were not observed in any of our studies.

Single topical applications of ETH50 as a large not micronized form or small particle dose form showed only limited absorption in vitro or in vivo. By comparison, the repeat topical dosing at a maximum tolerated ETH50 dosages to rats indicated the a.i. could be measured in plasma samples taken at different time points during the study. However, clear dose and duration of exposure relationships could not be established, even with differentiation of exposures to preclude oral ingestion of test item dosage formulation. In comparison to usual human topical exposures to a sunscreen product, rat dosages were up to 50 times higher as a.i./cm^2 and 200 times higher as particles/cm^2 in the 13-week dermal study. While the rat is not generally a good model for human topical tolerance, it can give useful information on systemic safety from repeated topical exposures. We also point out results from the in vitro percutaneous penetration study showing rat skin was relatively more permeable to ETH50 than human skin. This further supports the safety of ETH50 to be expected in humans given the lower skin penetration rate and expected lower systemic concentrations of a.i. from topical use of sunscreens containing up to 10% ETH50. Overall, the ETH50 should be considered as not bioavailable via oral or dermal exposure routes, which gives additional confidence to the conclusion for safety of the UV Filter.

Table 1. FAT 65'080 (ETH50) [Tinosorb A2B] In vitro Skin Penetration Results Comparison: 440 nm vs. 80 nm particle sizes
(Table corrected 19 April 2010)

Studies conducted in August 2005 and April 2007 followed same study design, under same conditions, had same source of ^{14}C-ETH50, and used same perfusate in the receptor chamber [6 % (w/v) polyethoxyoleate (PEG 20 oleyl ether) dissolved in physiological saline (0.9% NaCl w/v)]. Skin membranes were from different donors in the separate studies.

	Rat Skin Membrane (RCC A00112) Aug '05		**Rat Skin Membrane** (RCC B23624) April '07	
Mean Particle Size	**440 nm**		**80 nm**	
Applied Dose [µg/cm^2]	1912		1971.7	
Applied Volume [µL]	13		13	
Application Area [cm^2]	0.64		0.64	
Concentration [mg/cm^3]	94		97	
% of Dose Recovered	95.5		98.2	
In Receptor Fluid at:	% of dose	µg/cm^2	% of dose	µg/cm^2
6 h	0.05	0.972	< 0.01	*0.193
12 h	0.08	1.550	0.02	*0.321
24 h	0.12	2.246	0.02	*0.486
Flux [µg/cm^2/h]	0.209		0.035	
Amt Absorbed (% of Dose)	5.07		1.38*	
% in Skin Rinse	70.6		82.7	
% in Tape strips	16.0		13.4	

	Human Skin Membrane (RCC A00112) Aug '05		**Human Skin Membrane** (RCC B23624) April '07	
Mean Particle size	**440 nm**		**80 nm**	
Applied Dose [µg/cm^2]	1912		1971.7	
Applied Volume [µL]	13		13	
Application Area [cm^2]	0.64		0.64	
Concentration [mg/cm^3]	94		97	
% of Dose Recovered	91.8		98.9	
In Receptor Fluid at:	% of dose	µg/cm^2	% of dose	µg/cm^2
6 h	0.04	0.811	< 0.01	*0.150
12 h	0.07	1.375	< 0.01	*0.246
24 h	0.10	2.004	0.02	*0.373
Flux [µg/cm^2/h]	0.178		0.026	
Amt Absorbed (% of dose)	0.28		0.06	
% in Skin Rinse	73.1		94.5	
% in Tape strips	15.4		3.7	

* value calculated from the measured dpm, but most of the measured dpm were below LOQ

Damaged Skin. In answer to questions on skin penetration changes in "damaged skin," we present here in preview our draft study results from an in vitro percutaneous penetration study with tape stripped human skin subsequently exposed to micronized 14C-ETH50. The human cadaver skin was tape stripped 3 times, dermatomed and then circular samples cut for mounting in a flow-through apparatus as used in our studies presented above. The dosing formulation was the same one as that

was used in our study (Ref. 2) and here it was applied at a dose of 2053 µg a.i./cm^2 for 24-hours without rinsing. Results after 24 hours showed a wide difference between cells (large standard deviation of mean) but a low overall penetration (perfusate plus remaining skin membrane) of 0.76% (SD± 1.67) of applied dose. A full study report will be submitted after it has been finalized.

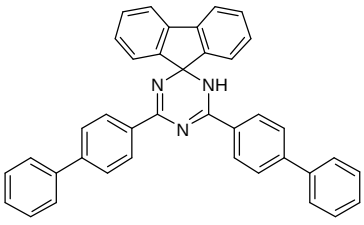
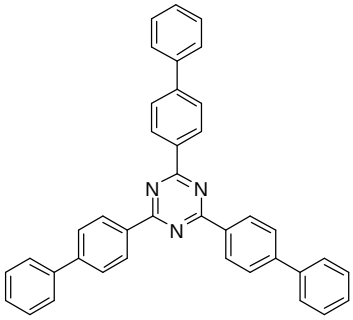
While a standard definition of "damaged skin" is not available, we have taken this approach as a first approximation of ETH50 absorption when applied to damaged skin. The variances can be interpreted as representative of the different effects of tape stripping by a non-standardize method and can be considered representative of compromised skin, mainly stratum corneum, as might occur in humans with a compromised integument. As such, even though the percutaneous penetration amount is about 10-fold higher than "normal skin", the amount absorbed remains below 1% of applied dose, an amount that could also represent impurities present in the dosing formulation, as discussed above under the ADME study. Our conclusion is that micronized ETH50 on damaged human skin is not absorbed at a rate meaningfully higher than on normal skin.

From the collected studies with ETH50, either as not micronized or micronized particle size, it is clear that the active ingredient – ETH50- does not penetrate skin, does not accumulate in body tissues, and is readily eliminated from circulation or intestinal tract after ingestion. The most apparent trends of measured radiochemical could be fully justified as due to by-products or impurities in the test item dosage formulation.

ETH50 Isomer. Presence of an structural isomer of ETH50 became most apparent in the plasma samples of rats given oral gavage doses in the UDS assay. This isomer was not found in other animal studies, including the mouse micronucleus in vivo and rat 13-week dermal studies. A short investigation to assess a biological origin of this isomer was conducted as in vitro chemical extraction test with ETH50 not micronized (FAT 65'080/B) and micronized (FAT 65'080/F) under either acidic or alkaline conditions (Wieser 2008; Ref 11). The two extraction conditions were intended as rough approximations of stomach and intestinal conditions following an oral dosing to see if the isomer was formed under either condition. In both situations, the ETH50 isomer was not found at higher concentrations than present in the original test item samples.

The detection of this isomer, its structure compared to ETH50 is shown in Figure 1, became noticeable because the plasma analytical method sensitivity was increased by LC-MS instrumentation that was not used in earlier studies and because the isomer was the principally occurring substance found in the plasma samples. As documented and discussed above, the isomer was also present in all batches of ETH50 used in all safety studies and is a part of the product placed on the market. We find the safety aspects of this isomer are not in question because adverse effects did not occur in any of the previously summarized studies for ETH50.

Figure 1. Isomer (left) and ETH50 (right) structures

 Molecular Weight =537.67 Exact Mass =537.22 Molecular Formula =C39H27N3	 Molecular Weight =537.67 Exact Mass =537.22 Molecular Formula =C39H27N3
4',6'-Bis-biphenyl-4''-yl-1'2'-dihydro-spiro[9H-fluorene-9,2'-[1,3,5]triazine]	1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-

Pro-inflammatory Effects. ETH50 is anticipated to be used in spray-on sunscreen formulations and accordingly we have conducted a single exposure inhalation toxicity study in rats. In recognition of the potential inhalation hazard and unknown effects of small particle size ETH50 on lung response the study design also assessed the inflammatory response markers in lavage fluid of exposed rats. In the study results of ETH50-exposed compared to placebo group responses, the ETH50 rats showed a reversible lung cellular response typical for exposure to particulate materials but without sensitization or immunological components.

Additional evidence that ETH50, regardless of particle size, does not present a severe inflammatory or immunological hazard, we refer to the full body of non-clinical study results and do not find evidence of immunologic challenges or adverse effects. The inflammatory and pro-inflammatory responses did not occur in any other tissues of other safety studies we have conducted with ETH50, regardless of particle size. Intestinal mucosa, lymphoid system, and circulating cellular components did not show any responses that would be characterized or indicate adverse effects of this substance. Photo-sensitization did not occur and dermal phototoxicity or pre-neoplastic changes did not occur in hairless mice exposed to micronized ETH50. The absence of immune responses in standard sensitization assays further supports ETH50 as a non-immunogenic substance.

Accordingly, we find ETH50 suitable for use in spray-on sunscreen products based on the absence of respiratory immunological responses and a lung inflammatory response indicative of normal response to particle loading to the lungs. The inflammatory response was seen at respirable particle sizes of ETH50 but not the placebo as used in our present study (~1 µm MMAD); however, exposure to such an aerosol size distribution is not expected from sunscreen spray applicators because they are designed to deliver sprays with aerosol particle sizes above 30µm diameter (Durand et al. 2007). These sunscreen spray applicators will deliver sprayed droplets at diameters 30-fold larger than those used in our rat inhalation study; droplets which are not expected to reach the upper airway or deeper respiratory system but impact or adsorb in the nasopharyngeal regions. Further, any adsorbed spray droplets containing ETH50 will, based on our completed safety studies, have limited bio-availability and if absorbed systemically will not have adverse effects or accumulate in the body. While ETH50 in a respirable aerosol induced a normal inflammatory response of the lung, this was reversible, presumably by clearance mechanisms, and without adverse pathology. We consider micronized ETH50 to be safe for use in sunscreens and other cosmetic products with spray-on applicators.

4. SAFETY EVALUATION- RISK ASSESSMENT

Given the particulate nature of the UV Filter ETH50 and the inclusion of nanometer sized particles (diameter <100 nm) in the marketed product, we consider the safety characterization should include both particle and soluble chemical exposures from topical application of cosmetic products. The collective non-clinical study results did not show any new toxicity effects from micronized ETH50 when compared to not micronized ETH50. Systemic concentrations of ETH50 in plasma did not show a clear dose or time relationship by oral gavage or dermal dosing, except from an intraperitoneal dose to mice, which is an unreasonable to impossible human exposure route for even a foreseeable worst case exposure scenario and is therefore not considered in our safety assessment.

Throughout this Addendum we presented animal dosages as mass of ETH50 and also as equivalent exposures of particle number and surface area. As a relative perspective on human skin exposures to particles of micronized ETH50, we use a 10% ETH50 (a.i.) concentration applied in sunscreen at 2 mg/cm^2 to 17.5 m^2 surface area of a 60 kg body weight human. Exposures as particle parameters were calculated from our characterization data given in the introduction to this addendum.

As shown in the next Table 1, compared to the rat topical doses used in the 13-week subchronic study, the key parameter of dose per skin surface area gives a margin of exposure (MoE) for humans that is about 50 times lower than applied daily to rats. If the commonly accepted application rate of 1 mg sunscreen/cm^2 is used the MoE ratios would double and approximate 100.

Table 1.	% a.i. applied	mg a.i./ cm^2 on dose site	Number a.i. Particles/ kg b.wt./d	Number a.i. particles/ cm^2	Specific surface area (m^2) dosed/cm^2
Rat 13-wk dermal: 1000 mg a.i./kg bwt./d	40	10	2.31E+15	2.31E+13	0.549
Estimated Human exposures	10	0.2	6.93E+13	4.6E+11	0.011
MoE (rat / human)	**4**	**50**	**~33**	**50**	**50**

We consider this margin of exposure sufficiently conservative in light of all of the previously reviewed safety studies with a full range of ETH50 particle sizes and dosages. We did not see any new or different types of adverse effects with micronized ETH50; in fact, the in vitro dermal absorption rate of ETH50 as a worst case particle size of about 80 nm on normal skin was about 10-fold lower than that from larger-sized (6 µm) micronized ETH50. Even application to damaged skin in vitro did not give a meaningful increase in absorption of ETH50 compared to normal skin. We consider ETH50 not to be biologically available after dermal or oral exposures.

Margin of Safety. While we consider ETH50 not to be absorbed across skin or absorbed systemically to an meaningful extent, the calculation of a Margin of Safety is then an unnecessary activity. However, given the tendency to put some numbers around the estimation and risk characterizations, we will make rote calculations for an MoS.

Taking the standardized approach to risk characterization, we compare systemic estimated human exposures that could be assumed to result from 10% ETH50 in cosmetic product applied as a 80nm particle to normal skin, as a 80nm particle on damaged skin, and as a 6 µm particle to normal skin. When data were available, we have used 3 different absorption functions to approximate dermal kinetics for the a.i.: % of applied, µg/ cm^2, and flux at steady state. We reiterate our opinion that unavoidable radiolabelled impurities have contributed to some of the absorption values given the exceedingly low bioavailability of ETH50.

Using the available data for ETH50 (FAT 65080) the estimated Margin of Safety calculations are summarized in Table 2.

Table 2.		**Comparative MoS calculations for ETH50** [Based on human skin in vitro study results for micronized **(80 nm) particle size on Normal skin**]		
Parameter		**% of Applied**	**Est. Absorption**	**Flux**
Adult Body weight (Kg)		60	60	60
Body surface area (cm^2)		17.500	17.500	17.500
Sunscreen applied (g) (if at 1 mg/cm^2)		18	18	18
FAT 65080 applied (mg) (10% formulation)		1800	1800	1800
Absorption function		0.06% of applied	1.2 $\mu g/cm^2$	0.026 $\mu g/cm^2/h$
Duration of exposure		1 day	1 day	6-hours
Systemic Estimated Dose (mg/kg/day)		0.018	0.35	0.046
NOAEL (mg/kg/day) Rat 13-wk oral study		1000	1000	1000
Normal skin: MoS (NOAEL/SED)		**55,555**	**2800**	**21,978**
80 nm median Particle diameter (2008 draft results)				
Damaged skin	**Absorption**	0.8% of applied	Not Available	Not Available
	MoS	**4166**	Not Available	Not Available
6 µm median particle diameter (2005 dossier)				
Normal skin	**Absorption**	0.2% of applied	5.4 $\mu g/cm^2$	0.178 $\mu g/cm^2/h$
	MoS	**1667**	**636**	**3225**

These comparative calculations each show ETH50 exceeds the accepted MoS of 100 for human exposure to UV filters and that it can be considered safe for topical use in humans at concentrations even above a 10% incorporation rate in a sunscreen applied to normal or damaged skin. We extend this safety assessment to include spray-on cosmetic products because the spray aerosol is not expected to contain significant amounts of respirable sized components and any inhaled spray would be adsorbed in the nasopharyngeal region with out adverse local or systemic effects.

Our risk calculations are extremely conservative because the absorption functions are based on many values that were below analytic quantification levels. Measurable amounts of ETH50 such as plasma and blood AUC estimates could have also been due to labelled impurities in excess of ETH50. The non-clinical dermal studies used dosages, as mass a.i., number of a.i. particles, or surface area, that were at or up to 50 or 100 times higher than estimated human topical exposures to ETH50. Additionally, the micronized particle size ($d_{0.5}$ 80 nm) used for the in vitro percutaneous penetration study was a worst case exposure because the marketed product will have a $d_{0.5}$ ~100 to 110 nm.

Thus, it is our opinion that any impacts of even the smallest sized particles in ETH50 have been addressed in our studies and safety assessment.

5. CONCLUSIONS

Based on the toxicology test results, exceedingly low bioavailability of the molecule, and margins of safety of 600 and greater for not micronized ETH50 and 2800 and greater for micronized ETH50, it is concluded that ETH50, micronized to product specifications, should be considered safe for use on normal or damaged skin at concentrations of at least 10% in sunscreen products and cosmetics of any application format including spray-on products.

6. REFERENCES

Analytical Information.

(A) Ciba Grenzach GmbH, Analytics, D-79630 Grenzach-Wyhlen. Analytical Certificate with FAT 65080/D. Dr. Edith Wieser (23.07.08)

(B) Ciba Grenzach GmbH, Analytics, D-79630 Grenzach-Wyhlen. Analytical Certificate with FAT 65080/F. Dr. Edith Wieser (23.07.08)

(C) Ciba AG, Trace Analysis & Issue Analytics, CH-4002 BASEL. Quantification of ETH-50 2nd Peak – Order Number 08.183. Denis Hundsbuchler (31 July 2008)

(D) Ciba Grenzach GmbH, D-79630 Grenzach-Wyhlen. Identity and Characterization of the Sample FAT 65080/E. Dr.Bernd Herzog (13.02.2007).

(E) Ciba Grenzach GmbH, D-79630 Grenzach-Wyhlen. Identity and Characterization of the Sample FAT 65080/F. Dr.Bernd Herzog (28.02.2007).

(F) RCC Ltd Environmental Chemistry & Pharmanalytics CH-4452 Itingen/Switzerland Determination of the particle size distribution of FAT 65080/B. Study number 859228; 09 September 2005

Toxicity tests and Supporting Literature.

1. CIT 2007-a. Acute oral toxicity in rats, acute toxic class method. CIT study no. 32808 TAR; 11 September 2007.
2. RCC 2007-a. [14C] FAT 65080 (ETH50) Percutaneous Penetration of [14C] FAT 65080 (ETH50), Micronized According to Technical Specifications, Through Rat and Human Split-thickness Skin Membranes (in-vitro). RCC study no. B23624; 1 November 2007.
3. RCC 2007-b. [14C] FAT 65080 (ETH 50): Absorption, Distribution and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. B23613; 1 November 2007.
4. RCC 2007-c. [14C] FAT 65080 (ETH 50): Absorption, Distribution, Metabolism and Excretion of [^{14}C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. A89280; 4 July 2007.
5. RCC 2007-d. 4-Hour Acute Inhalation Study in Rats with FAT 65'080/F. RCC study no. B38698. 16 November 2007
6. CIT 2008-a. Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. CIT study no. 32799 RSR. 30 July 2008.
7. CIT 2008-b. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008.
8. CIT 2007-c. Bone Marrow Micronucleus Test by Intraperitoneal Route in Mice. CIT study no. 32753 MAS. 16 June 2008.
9. CIT 2007-d. In vivo/in vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with FAT 65'080. Institute Pasteur de Lille report no. FSR-IPL 070101. 16 June 2008.

10. CRL-2007-e. Thirteen-Week Topical Range-Finding Study of FAT65'080/E in Hairless Mice, With or Without Simulated Sunlight. Charles River Laboratories Preclinical Services, Horsham, PA, USA; study number XAW 00001; 13 June 2007.

11. Wieser, E. 2008. Report on Analytical Test no.: 08/0662. Ciba Chemicals, PA Research & Technology Analytics. 29 July 2008.

7. ADDENDUM: SUMMARY OF THE PERFORMED TOXICITY TESTS

7.1. ACUTE ORAL TOXICITY

TYPE OF TEST :	**ACUTE ORAL TOXICITY TEST** OECD 423, 96/54/EC Annex V, B.1 ter
PERFORMING LABORATORY :	CIT BP 563 F-27005 Evreux France
GLP COMPLIANCE STATEMENT	Available (signed 11/07/2007).
QAU STATEMENT :	Available (signed 11/07/2007).
DATE OF TEST :	30 Jan - 22 Febr 2007.
TEST SUBSTANCE :	Micronized FAT 65'080/E, Batch KRG328-2 containing 49.5% ETH50 from lot number 11106CL4AA. Later analysis showed 51.4% purity. Micronized particle size: $d_{0.5}$=81nm
TEST ANIMALS :	Female Sprague-Dawley, rats.
DOSES TESTED :	2000 mg/kg.
RESULT :	LD_{50}-oral-rat > 2000 mg/kg .
OBSERVATIONS :	slightly reduced body weight gain in 1/6 females between day 1 and day 8.
REMARK	

7.2. IN VITRO PERCUTANEOUS PENETRATION

TYPE OF TEST :	***IN VITRO* PERCUTANEOUS ABSORPTION** OECD 428
PERFORMING LABORATORY :	RCC Ltd Environmental Chemistry & Pharmanalytics CH-4452 Itingen - Switzerland
GLP COMPLIANCE STATEMENT	Available (signed 01/11/2007).
QAU STATEMENT :	Available (signed 01/11/2007).
DATE OF TEST :	17 April – 10 May 2007.
TEST SUBSTANCE :	Micronized FAT 65'080, Batch KOC00050/004E, purity of 98 % [14C] labeled FAT 65'080, Batch number 49336-1-61, radiochemical purity ≥99% and specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg). Micronized particle size: $d_{0.5}$=80 nm; 91 nm
TEST SYSTEM :	Split-thickness skin (stratum corneum + viable epidermis), originating from full-thickness human skin (obtained post mortem; ages and body region of the donors are cited); rat skin.
DOSES TESTED :	2 mg/cm²
OBSERVATIONS :	**Rat skin membranes:** 24 hours after application only 0.02% of applied dose penetrated through the skin. Estimated flux 0.052µg/cm²/h (most values below LQ). End of exposure 83% of applied dose washed off from skin membranes. **Human skin membranes:** 24 hours after application only 0.02% of applied dose penetrated through the skin. Flux 0.042µg/cm²/h. End of exposure 94.49% of applied dose washed off from skin membranes Penetration through rat split-thickness skin membranes was slightly higher than through human split-thickness skin membranes. Comparison of the penetration through rat and human skin membrane based on the flux values lead to human/rat ratio 1:1.2.
RESULT :	Micronized FAT 65080, applied to rat and human skin membranes, penetrated at an extremely low rate and to a very limited extent through the skin membranes (total absorption was 1.38 % and 0.06 % of the applied dose, or 27.2 µg-eq/cm² and 1.2 µg-eq/cm² for rat and human skin membranes, respectively).
REMARK	Stability of test item in application medium checked by HPLC and found to be acceptable.

7.3. IN VIVO ORAL ABSORPTION, DISTRIBUTION, AND ELIMINATION

TYPE OF TEST :	**[14C] FAT 65080 (ETH 50): ABSORPTION, DISTRIBUTION AND EXCRETION AFTER ORAL ADMINISTRATION TO MALE RATS** OECD 417
PERFORMING LABORATORY :	RCC Ltd Environmental Chemistry & Pharmanalytics CH-4452 Itingen - Switzerland
GLP COMPLIANCE STATEMENT	Available (signed 01/11/2007).
QAU STATEMENT :	Available (signed 01/11/2007).
DATE OF TEST :	17 April – 25 May 2007.
TEST SUBSTANCE :	Micronized FAT 65'080, Batch KOC00050/004E, purity of 98 % [14C] labeled FAT 65'080, Batch number 49336-1-61, radiochemical purity >99% and specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg). Micronized particle size: $d_{0.5}$=80 nm; 91 nm
TEST SYSTEM :	Male rats, HanRcc:WIST (SPF): Wistar rats, outbred, SPF-quality.
DOSES TESTED :	100 mg/kg body weight
OBSERVATIONS :	After oral administration the extent of absorption from the gastro intestinal tract accounted for only 0.06 % of the administered dose. Almost the complete dose was excreted unabsorbed with the feces accounting for 93.34 % of dose within 48 hours after administration. Recovery averaged 93.56% of the applied doses during the study. The maximum concentration of radioactivity in blood was achieved 1 hour after administration, accounting for 0.360 µg FAT 65080 equivalents/g. This plateau level remained constant until 8 hours post dosing. Thereafter the concentration in blood decreased with a terminal half-life of about 31 hours. The AUC value was calculated to be 17.9 µg·h/g for blood. The tissue residues, 96 hours after administration, were very low. The highest concentration was found in abdominal fat but like all other selected tissues and organs the concentrations were all below the LOQ.
RESULT :	Micronized FAT 65080, administered to rats orally, was absorbed from the gastro intestinal tract only to a very low extent. Almost the complete dose was excreted unabsorbed with the feces. Residual concentrations in organs/tissues were all below the LOQ.
REMARK	

7.4. IN VIVO ORAL ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION

TYPE OF TEST :	**[14C] FAT 65080 (ETH 50): ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION AFTER ORAL ADMINISTRATION TO MALE RATS** OECD 417
PERFORMING LABORATORY :	RCC Ltd Environmental Chemistry & Pharmanalytics CH-4452 Itingen - Switzerland
GLP COMPLIANCE STATEMENT	Available (signed 04/07/2007).
QAU STATEMENT :	Available (signed 04/07/2007).
DATE OF TEST :	13 September –05 October 2007.
TEST SUBSTANCE :	Micronized FAT 65'080, Batch KOC00050/004E, purity of 98 % [14C] labeled FAT 65'080, Batch number 49336-1-61, radiochemical purity >99% and specific activity of 3676 MBq/mmol (99.34 mCi/mmol) or 6407 kBq/mg (173 µCi/mg). Micronized particle size: $d_{0.5}$ =6 µm
TEST SYSTEM :	Male rats, HanRcc:WIST (SPF): Wistar rats, outbred, SPF-quality.
DOSES TESTED :	100 mg/kg body weight
OBSERVATIONS :	After oral administration the extent of absorption from the gastro intestinal tract accounted for only 0.73 % of the administered dose. Almost the complete dose was excreted unabsorbed with the feces as unchanged parent, accounting for 97.2 % of dose within 48 hours after administration. The maximum concentration of radioactivity in blood and plasma was achieved 1 hour after administration, accounting for 2.463 and 4.359 µg FAT 65080 equivalents/g, respectively. This plateau level remained constant until 8 hours post dosing. Thereafter the concentrations in blood and plasma decreased with a half-life (8-48 h) of about 13 hours. The AUC values (0-96 h) were calculated to be 65.2 and 114.3 µg·h/g for blood and plasma, respectively. The tissue residues, 96 hours after administration, were very low. The highest concentration was found in abdominal fat accounting for 1.712 µg FAT 65080 equivalents/g. All other selected tissues and organs revealed concentrations below 0.110 µg FAT 65080 equivalents/g. The urinary metabolite pattern investigated revealed 7 metabolite fractions with the major fraction representing only 0.19 % of the dose. All other fractions were below 0.1 % of the dose.
RESULT :	Micronized 14C-FAT 65080, administered to rats orally, was absorbed from the gastro intestinal tract only to a very low extent. Almost the complete dose was excreted unabsorbed as unchanged parent with the feces. Residual concentrations in organs/tissues were very low.
REMARK	Radiolabelled impurities correlate to by-products in parent material or those present in radiochemical used.

7.5. ACUTE INHALATION TOXICITY

TYPE OF TEST :	**ACUTE INHALATION TOXICITY TEST** OECD 403
PERFORMING LABORATORY :	RCC Ltd Wölferstr. 4 CH-4414 Füllinsdorf - Switzerland
GLP COMPLIANCE STATEMENT	Available (signed 16/11/2007).
QAU STATEMENT :	Available (signed 16/11/2007).
DATE OF TEST :	11 September – 25 September 2007.
TEST SUBSTANCE :	Micronized FAT 65'080/F, Batch 04122FC7, containing 47.6% ETH50 from lot number 37874FC6.
TEST SYSTEM :	Wistar rat.
CONCENTRATION TESTED :	4.976 mg micronized ETH50/L air or 2.388 mg ETH50/L air per analytical determinations; MMAD 1.2 µm ± ~2 µm GSD Equivalent particle exposure: 1.2E19 a.i./L air Doses were at Maximum acheivable exposure concentration for 4-hours
OBSERVATIONS :	No premature deaths, no clinical signs and no macroscopic pathology findings; transient slightly lower group mean body weight three days after exposure to the test item. Findings in test item group: - on test day 2, lung weights and lung to terminal body weight ratios moderately higher than in placebo animals - by test day 15, mean lung weight only slightly higher in males - BALF (approximately 14 hours post end of exposure): total cell count (mainly macrophage and neutrophils; no lmyphocytes), TNFα and total protein considerably higher than in placebo animals; IL6 not consistently increased. - Microscopic pathology (~24 hours post end of exposure): granulocytic infiltration in alveolar wall and lumen, diffuse alveolar histiocytosis and alveolar lining cell activation - by test day 15, histopathology findings no longer evident. Histopathology findings were not seen in placebo control animals.
RESULT :	LC_{50}-inhalation-rat >4.976 mg micronized ETH50/L air or >2.388 mg ETH50/L air. Reversible inflammatory response considered representative of lung clearance of particle loading.
REMARK	Inhalation exposure to 1 µm aerosol from commercially used spray applicators is not expected.

7. 6. COMBINED REPEATED DOSE ORAL TOXICITY WITH REPRODUCTION-DEVELOPMENTAL TOXICITY SCREENING

TYPE OF TEST :	**COMBINED REPEATED DOSE ORAL TOXICITY STUDY IN RODENTS WITH REPRODUCTION-DEVELOPMENTAL TOXICITY SCREENING** OECD 422
PERFORMING LABORATORY :	CIT BP 563 F-27005 Evreux France
GLP COMPLIANCE STATEMENT :	Available (signed 30/07/2008). Analytical chemistry aspect not GLP-compliant; in preparation.
QAU STATEMENT :	Available (signed 30/07/2008).
DATE OF TEST :	27 February 2007 – 05 May 2007.
TEST SUBSTANCE :	Micronized FAT 65'080/F, Batch 04122FC7, containing 47.6% ETH50 from lot number 37874FC6. Later analysis showed 51.4% purity; Micronized particle size: $d_{0.5}$=109nm
TEST SYSTEM :	Rats of the Sprague-Dawley strain [Crl CD® (SD) IGS BR, COBS-VAF®]. - 10 males and 10 females per test group - two control groups received either the vehicle, purified water, alone, or the placebo alone.
DOSES TESTED :	0 - 100 - 500 – 1000 mg/kg/day as active ingredient. Total no. ai. particles dosed: 0, 0, 1.2E20, 5.8E21, 2.3E21 Dosage volume 5mL/kg; by oral gavage.
OBSERVATIONS :	No test article-related effects noted at the endpoints assessed except findings noted in the lungs secondary to the aspiration of formulation in 2/5 females of the high dose group. Lower mean body weights, mean body weight gains and mean food consumption noted in placebo control females when compared to vehicle control females.
RESULT :	NOAEL = 1000 mg/kg/day.
REMARK	Dose selection based on 13-week toxicity oral gavage study with the non-micronized test material where the NOAEL was 1000 mg/kg/day.

7.7. MUTAGENICITY - IN VIVO MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST

TYPE OF TEST:	**BONE MARROW MICRONUCLEUS TEST** OECD 474, 67/548/EEC Annex V, B.12
PERFORMING LABORATORY:	CIT BP 563 F-27005 Evreux, France
GLP COMPLIANCE STATEMENT:	Available (signed 16/06/2008).
QAU STATEMENT:	Available (signed 16/06/2008).
DATE OF TEST:	13 February – 16 June 2008
TEST SUBSTANCE:	Micronized FAT 65'080/E, Batch KRG328-2 containing 49.5% ETH50 from lot number 11106CL4AA. Later analysis showed 51.4% purity. Micronized particle size: $d_{0.5}$=81nm Reference substance: not micronized ETH50 (FAT 65'080/B; batch KOC00050/004.E); 98% purity.
TEST SYSTEM:	Mice of the strain Swiss Ico: OF1 (IOPS Caw).
DOSES TESTED:	0, 500, 1000, 2000 mg/kg body wt.; 0, 253, 506, 1012 mg a.i./kg body wt. Reference item at 2000 mg a.i./kg Dosage volume 10 mL/kg body wt. Doses given by intraperitoneal injection.
RESULT:	The test substance is considered to be non-mutagenic in this assay.
OBSERVATIONS:	The test article did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse.
REMARKS:	A pre-experiment to define maximum tolerated doses is available. Achieved plasma levels of ETH50 showed bone marrow was exposed to ETH50 by from either test item or reference item. Response to micronized ETH50 is not different from non-micronized ETH50.

7.8. MUTAGENICITY - UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES

TYPE OF TEST:	**UNSCHEDULED DNA SYNTHESIS (UDS) TEST WITH MAMMALIAN LIVER CELLS IN VIVO** OECD 486, 67/548/EEC Annex V, B.39
PERFORMING LABORATORY:	Institut Pasteur de Lille 1, Rue du Professeur Calmette, B.P. 245, 59019 Lille Cedex, France
GLP COMPLIANCE STATEMENT:	Available (signed 16/06/2008). Analytical chemistry aspect not GLP-compliant; in preparation
QAU STATEMENT:	Available (signed 16/06/2008).
DATE OF TEST:	15/01/2007 to 29/08/2008
TEST SUBSTANCE:	FAT 65'080/E, contained 49.5% ETH50 of batch number KRG328-2 prepared by micronizing ETH50 LOT11106CL4AA. Later analysis showed 51.4% purity. Micronized particle size: $d_{0.5}$=81nm Reference substance: not micronized ETH50 (FAT 65'080/B; batch KOC00050/004.E); 98% purity.
TEST SYSTEM:	Male rats of the strain Fischer from Charles River France. 3 males per group. A reference item group received the non-micronized reference item. Two control groups received either the vehicle, purified water, alone, or the placebo alone.
DOSES TESTED:	- 1000 and 2000 mg/kg for the test item - 2000 mg/kg for the reference item - 1000 mg/kg for the placebo control group.
RESULT:	The test substance is considered to be non-effective in this *in vivo / in vitro* UDS system. Plasma analysis indicated only low amounts of ETH50 but quantifiable amounts of an isomer present in the test item; Exposure of target tissue is supported.
OBSERVATIONS:	The test item, placebo and reference item did not induce DNA damage leading to increased repair synthesis in the hepatocytes in treated rats.
REMARKS:	Doses were selected based on a preliminary toxicity test to define maximum tolerated doses. The viability of the hepatocytes was not affected due to the in vivo treatment either with the test article, placebo or reference item. Response to micronized ETH50 is not different from non-micronized ETH50.

7.9. 13-Week Phototoxicity study in hairless mice

TYPE OF TEST :	**13-WEEK TOPICAL RANGE-FINDING STUDY OF FAT 65'080/F IN HAIRLESS MICE WITH AND WITHOUT SIMULATED SUNLIGHT.** STUDY DESIGN IS RECOGNIZED AS BY U.S. FOOD & DRUG ADMINISTRATION FOR EVALUATION OF UV FILTERS AND PHOTOACTIVE AGENTS IN DRUGS OR DRUG PRODUCTS.
PERFORMING LABORATORY :	Charles River Laboratories Pre-clinincal services (Argus); Horsham, PA, USA.
GLP COMPLIANCE STATEMENT	13/06/2007
QAU STATEMENT :	13/06/2007
DATE OF TEST :	05July – 20 October 2006 (in-life phase)
TEST SUBSTANCE :	FAT 65'080/E (KRG328-2; purity 50.6% active ingredient) d(0.5)=81 nm
TEST SYSTEM :	albino hairless Crl:SKH1-hr mice
DOSES TESTED :	100 µl dose form/mouse daily, Monday thru Friday mg a.i./g dose form: 0, 25, 50, 100, 200 mg a.i./cm^2 skin: 0.1, 0.2, 0.4, 0.8 no. a.i. particles/cm^2 skin: 2.3E17, 4.6E17, 9.2E17, 18.4E17
EXPOSURE PARADIGM:	Solar simulated UV irradiation of 0 (vehicle), 600, or 1200 RBU per week with and without ETH50
OBSERVATIONS :	Clinical changes and skin responses as found; skin fold thickness; body weights; gross necropsy; histological examination not performed. Adverse effects of UV irradiation found at higher incidence in mice without ETH50- including edema formation, skin wrinkling and increased skin thickness; early deaths did not occur; gross changes not remarkable.
RESULT :	Up to highest ETH50 exposures (0.8 mg a.i./cm^2) increased UV-induced effects did not occur; more toxic forms of ETH50 are not induced by UV exposures.
REMARK	

7.10. 13-Week Dermal Toxicity study in Rat

TYPE OF TEST :	**REPEATED DOSE 13-WEEK DERMAL TOXICITY STUDY IN RODENTS OECD 411, 67/548/EEC Annex V, B.26**
PERFORMING LABORATORY :	CIT BP 563 F-27005 Evreux France
GLP COMPLIANCE STATEMENT:	Available (signed 01/08/2008). Analytical chemistry aspect not GLP-compliant; in preparation
QAU STATEMENT :	Available (signed 01/08/2005).
DATE OF TEST :	22 Feb. 2007 – 14 June 2007 (in-life phase)
TEST SUBSTANCE :	FAT 65'080/F, KRG328-2 (Lot 04122FC7; micronized) purity 98.5 ± 2 % Particle size: $d_{0.5}$= 109 nm
TEST SYSTEM :	Wistar rats,: - 10 males and 10 females per test group. - 5 males and 5 females added to untreated and placebo control and both high-dose groups for treatment-free recovery period. - 3 males and 3 females were added to each test group for toxicokinetics investigations.
DOSES USED :	0 (not treated), 0 (placebo), 150, 500, 1000, 1000 (with collar) mg a.i./kg/day. As mg a.i./cm^2 skin: 0, 0, 1.5, 5, 10, 10 As number particles a.i./cm^2 skin: 0, 0, 3.5E18, 1.2E19, 2.3E19, 2.3E19
OBSERVATIONS :	No unscheduled death or premature sacrifice during the study. No test article-related effects noted at the endpoints assessed except scabs at dose site 6/15 M and 5/15 F of high dose (no collar); reversible body weight gain reduced in all high dose M; treatment stress-related increased adrenal (F) and thymus (M) weights. Plasma a.i. concentrations up to 16 ng/ml but without dose- or duration of dosing-relationships.
RESULT :	NOAEL = 1000 mg a.i./kg/day.
REMARK	14-day dose range finding performed

APPENDIX I

Ciba Internal Report:

Characterization of the Sample [14C] FAT 65080 (Tinosorb A2B) with Respect to its Particle Size Distribution

6th December 2007

s47F
G-9001.2.28

Phone: s22
e-mail: s22 @cibasc.com

s47F
G-9001.2.21

Phone: Phone: s22
e-mail: s22 @cibasc.com

Characterization of the Sample [14C] FAT 65080 (Tinosorb A2B) with Respect to its Particle Size Distribution

Objective

For some toxicological studies with Tinosorb A2B, radioactively labeled (14C) material had to be used. This was the case with a study of percutaneous penetration, and a study on absorption, distribution and excretion after oral administration to male rats. Since Tinosorb A2B consists of sub-micrometer particles, the radioactively labeled UV absorber had to be processed in terms of particle size reduction in the same manner as the material, which is intended to be marketed and which has been employed in the other toxicological investigations. The UV absorbing material of Tinosorb A2B is called ETH50. The INCI name is Tris-Biphenyl-Triazine.

The purpose of this report is to show, that the properties of the dispersed radioactively labeled ("hot") UV absorber used for the above mentioned studies in terms of particle size distribution are the same as with the material which is intended to be marketed and which was used in the other toxicological studies. In order to do so, all samples were characterized with Fiber Optic Quasi Elastic Light Scattering (FOQELS). Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were applied only to the "cold" samples.

Experimental

Method 1: FOQELS

The particle size of this sample was measured with the FOQELS (Fiber Optic Quasi Elastic Light Scattering) technique. This is a dynamic light scattering technique, which is based on measurement of the intensity fluctuations of the back-scattered light of a dispersion of particles. In comparison to conventional goniometer-type light scattering measurements, measuring the back-scattered light has the advantage that the sample must only moderately be diluted (2% w/v of active), and extensive dust removal procedures can be renounced.

However, with goniometer measurements the data can be extrapolated to angle zero, where the form factor for all particle sizes is unity (there is no interference of scattered light at angle zero). But when measuring the back-scattered light, one sticks to a one-angle measurement. This is no problem with mono-disperse samples, but with poly-disperse particle dispersions as they are obtained from ball milling procedures, one has then to consider the form factor P(q, R). This quantity depends on the scattering angle (q is the scattering vector, which is derived from the scattering angle) and on the particle radius R. The volume weighted particle size distribution $F_V(R)$ is then given as

$$F_V(R) = \frac{G(R)}{R^3 \cdot P(q,R)}$$

where G(R) is obtained from evaluation of the scattering data with the CONTIN software [1]. The radius R is given as:

$$R = \frac{k \cdot T}{6 \cdot \pi \cdot \eta_0 \cdot D_q}$$

where η_0 is the solvent viscosity and D is the diffusion coefficient at scattering angle q.

With the distribution $F_V(R)$ it is possible to evaluate a median value of the particle size distribution:

$$\frac{\int_0^{R(0.5)} F_V(R)dR}{\int_0^{\infty} F_V(R)dR} = 0.5$$

and d(0.5) = 2·R(0.5).

The treatment of FOQELS measurements just described was validated by disc centrifugation studies [2] and confirmed by atomic force microscopy.

Method 2: Electron Microscopy

For Transmission Electron Microscopy (TEM) an *EM 910* (*Zeiss)* was used. For the sample preparation, the Tinosorb A2B dispersion was diluted with water, and after treatment in ultrasonic bath, placed on a TEM grid. Measurements were done after drying of the samples over night at ambient temperature. The conditions were: High vacuum < 10^{-6} mbar, electron acceleration voltage 100 kV, beam current 4 mA.

Scanning Electron Microscopy (SEM) was performed with a *Quanta 200 FEG (FEI Company)*. The conditions were: High vacuum < 10^{-6} mbar, sputtering of samples with Au/Pd in order to achieve the necessary conductance.

Description of the Samples

a) FAT 65080 (ETH50) was milled without mixing with radioactively labeled substance at a concentration of 30%. The resulting dispersion was designated as ***MUS686***. The particle size of this dispersion was measured by FOQELS and also with TEM and SEM. The sample was at least stable for six days (checked with FOQELS measurements).

b) FAT 65080 (ETH50) was mixed with the pure radioactively labeled [14C] FAT 65080 (ETH50). The mixing was performed by dissolving both in a suitable solvent, then stirring, and evaporating of the solvent afterwards. After that, the resulting mixture was milled in the same way as was done under a). The resulting 30% dispersion of [14C] FAT 65080 (ETH50) was split into two samples: ***MUS687*** and ***MUS688***. The particle sizes of the two samples were measured using FOQELS. For reasons of contamination, TEM and SEM measurements were abandoned in this case. However, since the chemical nature of the material and the milling process were the same for *MUS687*, *MUS688*, and *MUS686*, in case of equal FOQELS results of *MUS687* and *MUS688* compared to *MUS686*, the SEM and TEM pictures of *MUS686* would also be applicable to *MUS687* and *MUS688*.

c) Finally, for comparison of the particle size, a production sample was characterized using FOQELS, and TEM and SEM. This sample was ***Tinosorb A2B Lot 07612FC7***.

Results

Sample 686

Figure 1 shows the particle size distribution of the dispersion *MUS686*, based on the cold material FAT 65080 (ETH50). The particle size distribution was measured with FOQELS. The median value of the diameter d(0.5) is given as the average of 10 measurements.

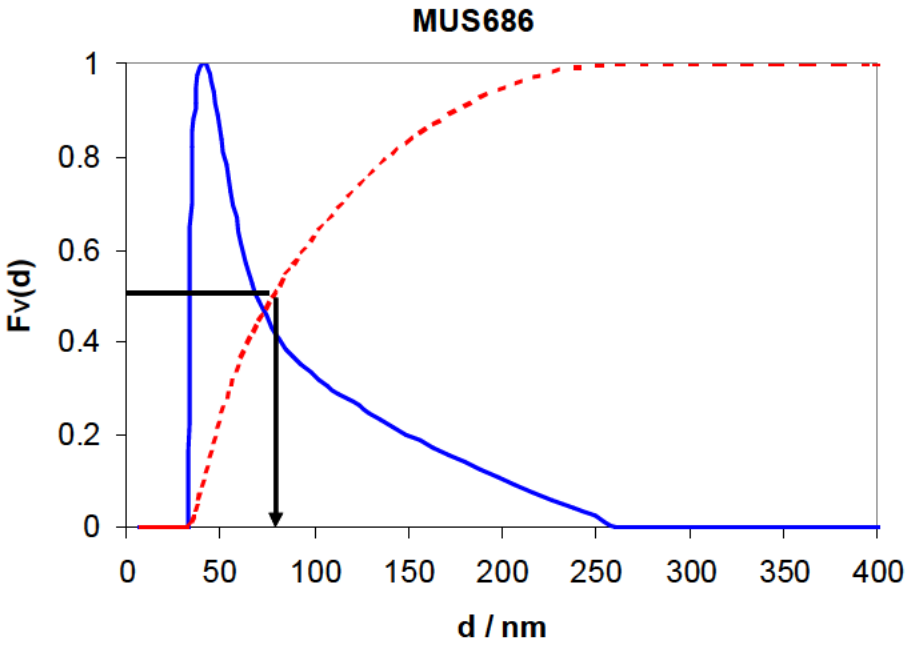


Figure 1: FOQELS result with the "cold" sample *MUS686* of the dispersion of FAT 65080 (ETH50); the blue solid line is the particle size distribution, the red dotted line is the cumulative particle size distribution, the black dotted lines indicate the determination of the d(0.5) value: **d(0.5) = 84 ± 13 nm**

In Figures 2 and 3 the corresponding TEM and SEM pictures of the dispersion *MUS686* are shown.

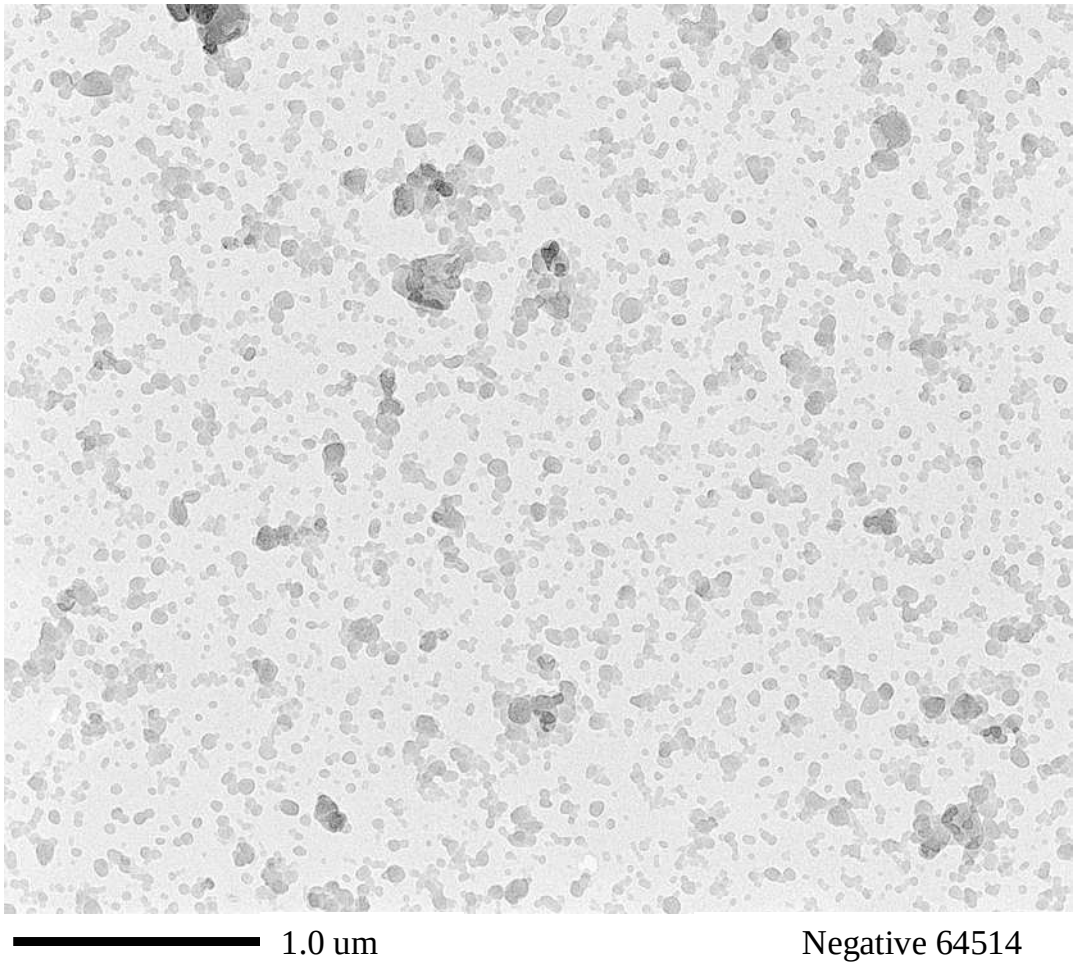


Figure 2: TEM picture of the ("cold") sample of the dispersion of FAT 65080 (ETH50) named *MUS686*

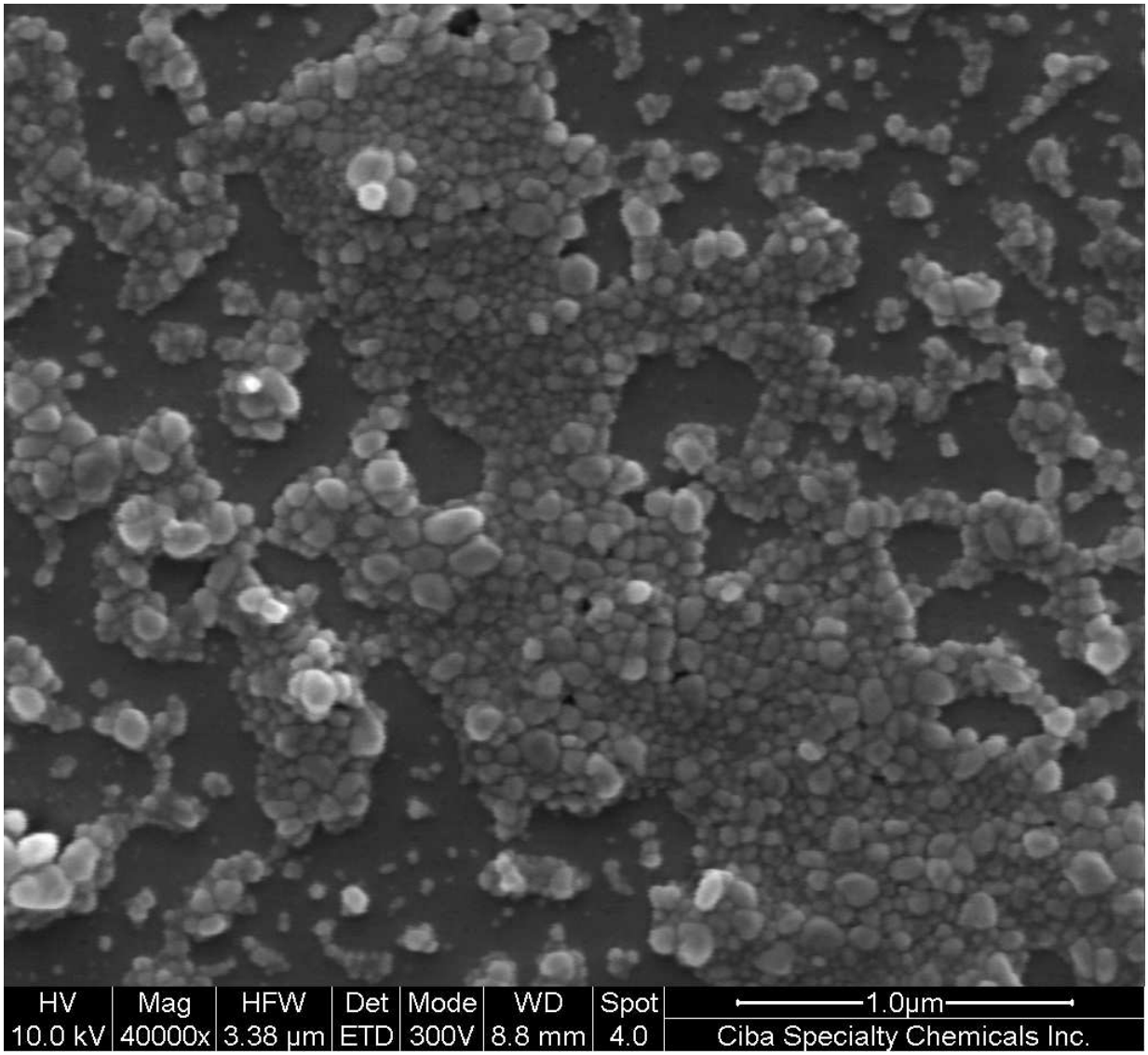


Figure 3: SEM picture of the ("cold") sample of the dispersion of FAT 65080 (ETH50) named *MUS686*

The electron micrographs of *MUS686* are in line with the FOQELS measurements (Figure 1) showing a size distribution of particles between 20 and 300 nm. About 60% of the solid phase of the dispersion is made up of particles smaller than 100 nm.

Samples 687 and 688

Samples 687 and 688 are dispersions of [14C] FAT 65080 (ETH50), and Figures 4 and 5 show the respective particle size distributions measured with FOQELS (averages of 10 measurments).

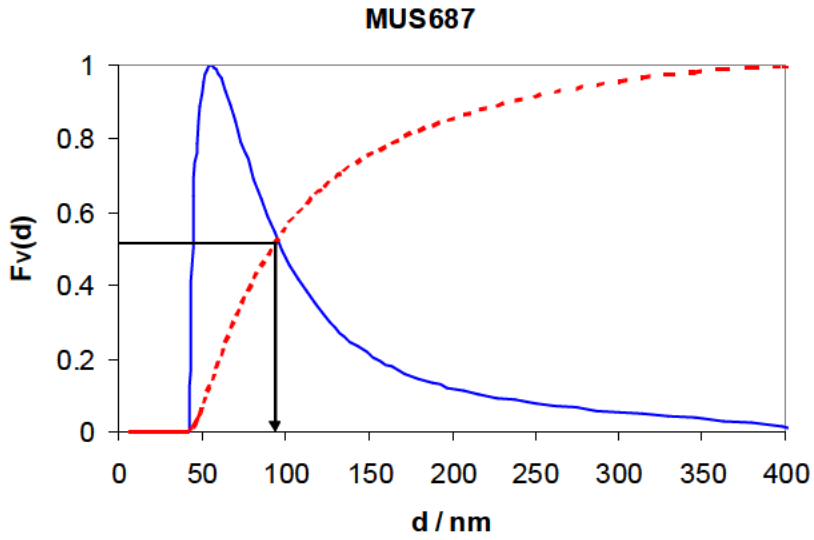


Figure 4: FOQELS result with the sample *MUS687* of the dispersion of [14] FAT 65080 (ETH50); the blue solid line is the particle size distribution, the red dotted line is the cumulative particle size distribution, the black lines indicate the determination of the d(0.5) value: **d(0.5) = 92 ± 25 nm**

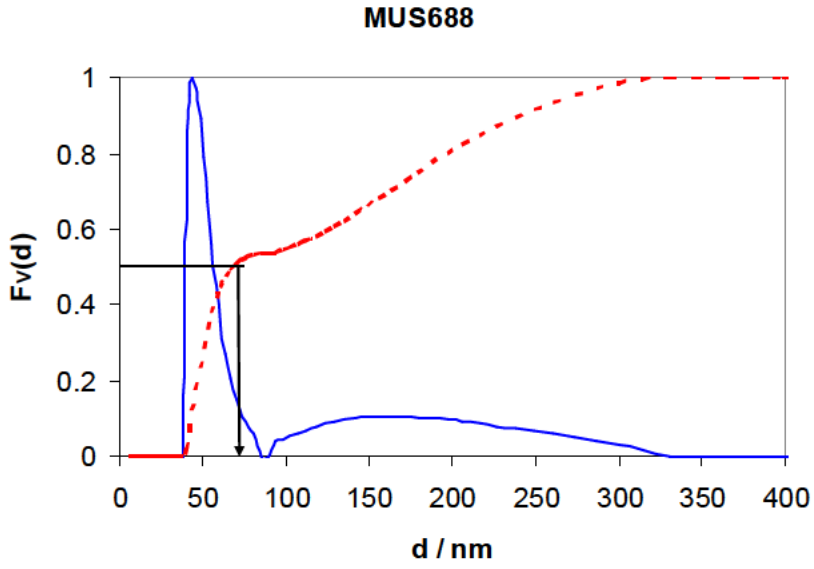


Figure 5: FOQELS result with the sample *MUS688* of the dispersion of [14] FAT 65080 (ETH50); the blue solid line is the particle size distribution, the red dotted line is the cumulative particle size distribution, the black lines indicate the determination of the d(0.5) value: **d(0.5) = 81 ± 28 nm**

Tinosorb A2B OP Lot 07612FC7

For comparison, a production batch (Tinosorb A2B OP Lot 07612FC7) was also investigated. Figure 6 shows the respective particle size distribution measured with FOQELS.

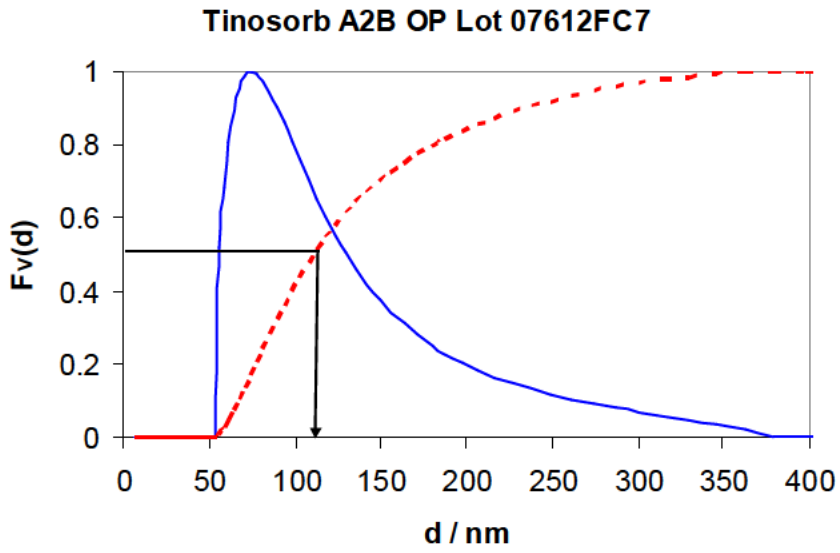


Figure 6: FOQELS result with a sample of Tinosorb A2B OP Lot 07612FC7; the blue solid line is the particle size distribution, the red dotted line is the cumulative particle size distribution, the black lines indicate the determination of the d(0.5) value:
d(0.5) = 110 ± 16 nm

Figures 7 and 8 show the TEM and SEM pictures taken with a sample of Tinosorb A2B OP Lot 07612FC7.

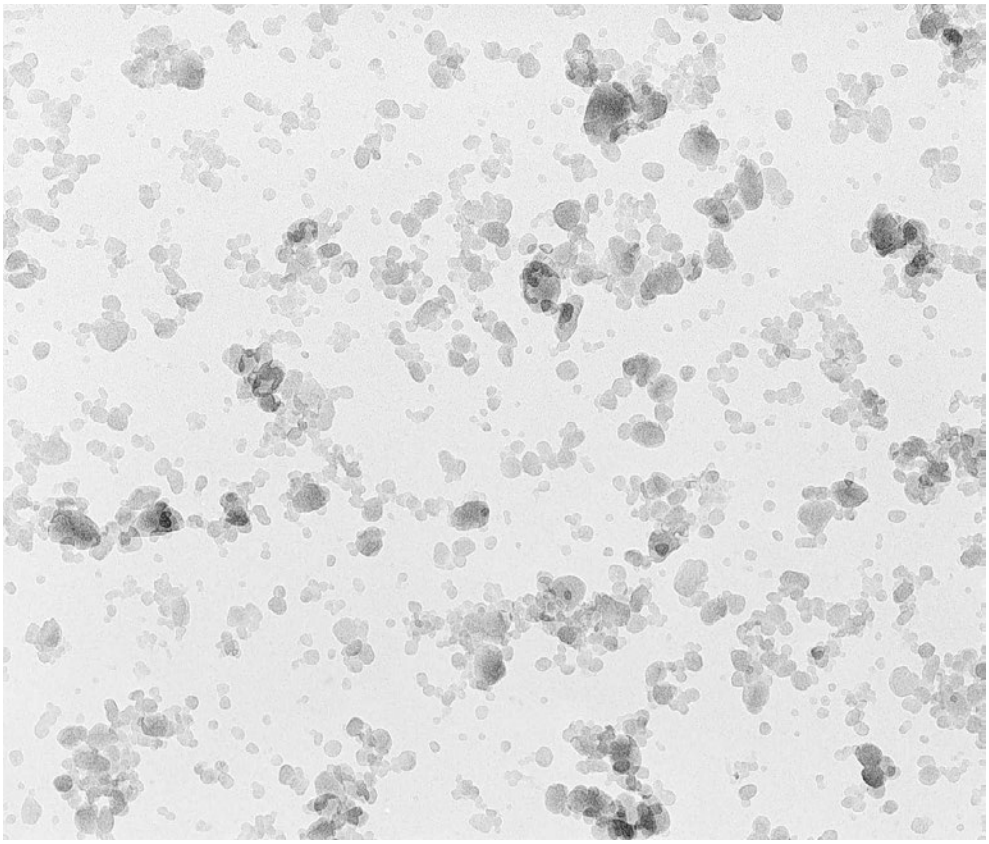


Figure 7: TEM picture of a sample of Tinosorb A2B OP Lot 07612FC7.

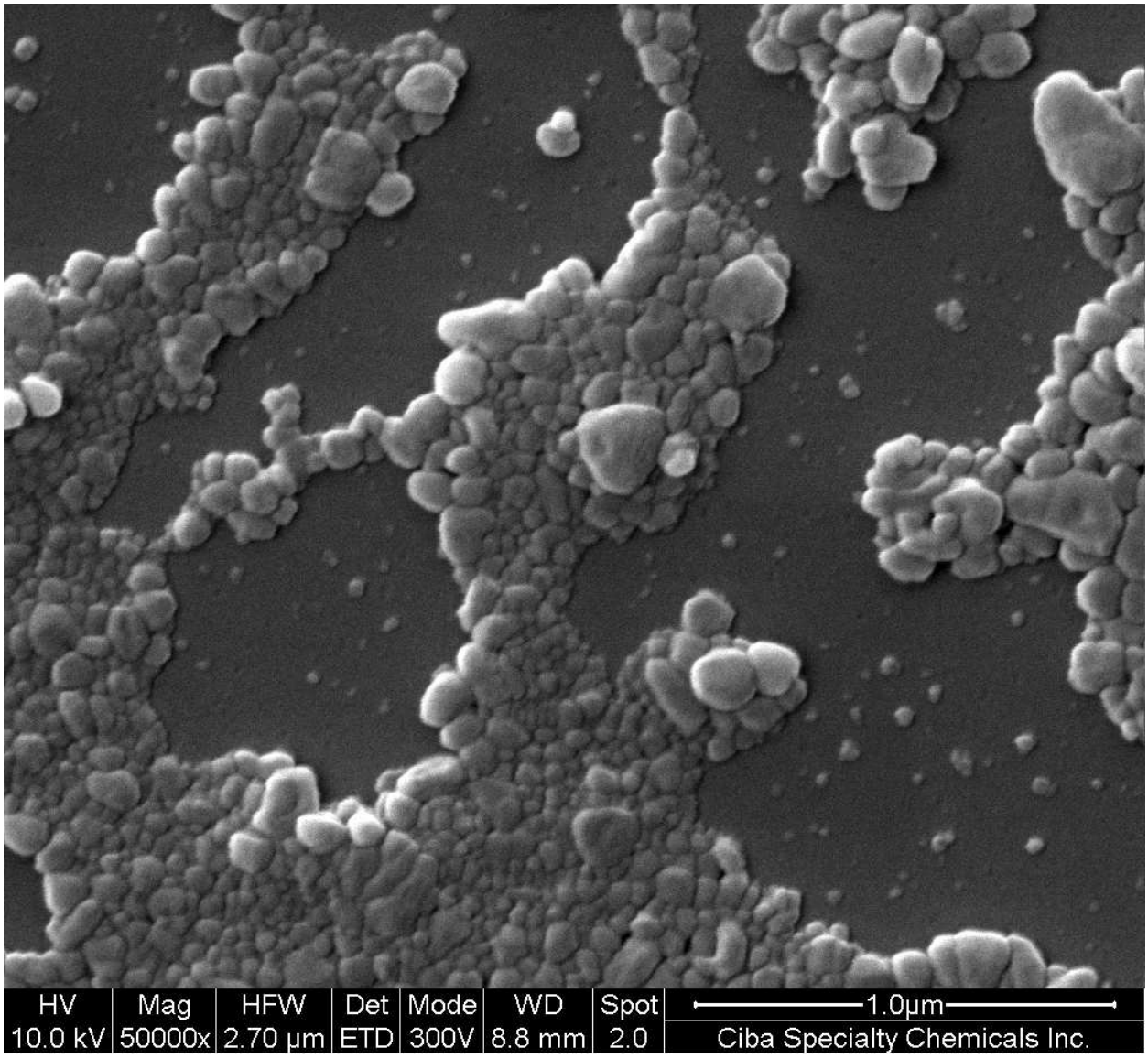


Figure 8: SEM picture of a sample of Tinosorb A2B OP Lot 07612FC7.

Conclusion

We have demonstrated in this report that the dispersions containing the radioactively labeled UV absorber ETH50 have equivalent properties in terms of particle size as the respective "cold" material and as a representative production sample (Tinosorb A2B OP Lot 07612FC7). It was also shown for the "cold" samples, that the results of the FOQELS measurements are consistent with transmission and scanning electron microscopic pictures of the respective dispersions. The results of the FOQELS measurements are summarized in Table 1.

Table 1: Results of FOQELS measurements

Sample	*d(0.5) / nm*
Sample 686 containing "cold" FAT 65080 (ETH50)	84 ± 13
Sample 687 containing [C14] FAT 65080 (ETH50)	92 ± 25
Sample 687 containing [C14] FAT 65080 (ETH50)	81 ± 28
Sample of Tinosorb A2B OP Lot 07612FC7	110 ± 16

The particle size of the production sample seems to be somewhat larger than that of the other samples, but the standard deviations around the average particle sizes of all samples are still overlapping. That means that the particle sizes of the four samples are the same within the range of experimental error.

References:

1. Provencher, S. W., Makromol. Chem. **180**, 201 (1979)
2. Herzog, B., Katzenstein, A., Quass, K., Stehlin, A., Luther H., J. Colloid Interface Sci., **271**, 136 – 144 (2004)

End of Appendix I report.

APPENDIX II

SAFETY ASSESSOR CERTIFICATION OF DOSSIER

Dossier: ETH50 as UV Filter for Sunscreen Products

This Dossier's Supplement has been prepared and reviewed by the undersigned and reflects accurately the testing results summarized herein to support the safe use of the product in cosmetics.

s47F
Toxicologist
s47F
s47F
Ciba, Inc.
Basel, Switzerland

s47F
Regulatory Affairs
s47F
s47F
Ciba, Inc
Basel, Switzerland

13 August 2008

BASF
The Chemical Company

SUPPLEMENT VI

TO THE TOXICOLOGY SUMMARY
And
SAFETY ASSESSMENT FOR ETH50 (FAT 65'080) as UV FILTER in SUNSCREEN PRODUCTS

(First Dossier Submitted on 08 November 2005 by Ciba AG)

Ciba AG is now part of BASF

ANSWERS TO QUESTIONS FROM THE "NANO" WORKING GROUP OF SCIENTIFIC COMMISSION FOR CONSUMER SAFETY AS RECEIVED IN JULY 2010

PREPARED BY
BASF SE
Ludwigshafen, Germany

07 January 2011

CONTENTS

BACKGROUND to SUPPLEMENT VI

In response to the review of additional technical and toxicology information in the Supplements III, IV, and V to the original ETH50 Dossier submitted in November 2005, the SCCS Expert Group on Nano Topics requested additional information on our UV Filter substance ETH50. In a meeting with DG SANCO and the SCCS Expert Group on Nano, it was asked to provide additional information on:

- the acute inhalation toxicity study
- the severity of the inflammatory response observed in the acute inhalation study
- exposure to ETH50 when used in spray formulations
- the MoS calculation for inhalation
- the MoS calculation for the dermal route
- the possibility for accumulation

With this Supplement VI we are pleased to have the opportunity to submit our answers and additional information that we have available currently.

This Supplement will in separate sections present the question or request followed by our answer and available information.

REQUESTED INFORMATION

I. Further information on the inhalation toxicity and respiratory response.

A. Question on the actual dose used in the acute inhalation study

The acute inhalation toxicity of FAT 65'080/F was assessed when administered to rats for a single 4-hour period (RCC 2007a). Details of the applied test procedure and the obtained results can also be found in submission III (13 August 2008, revised version 07 January 2010). In the following section, we will explain more in detail the test atmosphere conditions and the achieved test concentrations. In general, the study was designed to comply with OECD test guideline No. 403 (1981), and with US EPA guideline OPPTS 870.1300 (1998). In addition, the study examined lung inflammatory response markers via broncho-alveolar lavage fluid (BALF) sampling.

The test item, FAT 65'080/F, contained 47.6% ETH50 prepared by micronization with excipients decyl glucoside, silicon defoaming agent, xanthan gum and butylene glycol; particle median diameter was 109 nm ($d_{0.5}$). A placebo group was used to identify any effects related to the excipients. The test item and the placebo were prepared as aqueous dilutions at a ratio (w/w) of 20% FAT 65'080/F or 20% placebo plus 80% purified water, **so the target concentration as active ingredient of UV filter ETH50 was 10%**. Both the placebo and the test item aerosols were generated at ambient conditions using a cyclone glass atomizer that was operated at maximum throughput. Therefore, the aerosol concentrations administered to the animals were considered to represent the highest technically achievable aerosol concentration levels. The concentrations of the placebo and test item dilutions were determined

gravimetrically and/or by chemical analysis, the particle size distribution was determined gravimetrically.

Rats (15 per sex) were exposed by nose-only to an aqueous dilution of FAT 65'080/F at a chemically determined mean aerosol concentration of 4.976 mg/l air (s.d. ± 0.513 mg/l air), equivalent to a gravimetrically determined mean concentration of 0.602 mg/l air (s.d. ± 0.059 mg/l air). In appendix I of the report (pages 40-50), the achieved concentrations in the test chamber for the placebo and test item group, the particle sizes, as well as the Mass Median Aerodynamic Diameters can be found.
Two gravimetric measurements of particle size distribution during exposure indicated mass median aerodynamic diameters (MMADs) and geometric standard deviations (GSD) of 1.23 µm (GSD 2.13) and 1.27 µm (GSD 2.19). The placebo group was exposed to an aqueous dilution of the control item, at aerosol generation conditions similar to those used for the test item group. Two gravimetric measurements of particle size distribution during this exposure produced mass median aerodynamic diameters (MMADs) and geometric standard deviations (GSD) of 1.08 µm (GSD 2.25) and 1.10 µm (GSD 2.27).

The Mercer impactor data on page 43 and page 44 for the placebo group give a cumulative mass of 357 and 381 µg during a 4-minutes sampling at a flow rate of 1 L/min which corresponds to about 90 µg/l. This value is in excellent agreement with the gravimetrical measurements of 91 µg/l (see page 41-43). The Mercer impactor data for the test group 2 are given on page 48 and 49 of the RCC report. A cumulative mass of 462 µg und 516 µg during a 1-minute sampling at a flow rate of 1 L/min resulted in a concentration of around 500 µg/l of active ingredient. This again is in excellent agreement with the analytical concentration of 4.976 mg/l formulation containing 10 % active ingredient. The lung burden was calculated to be about 1.17 mg per animal assuming a MMAD of 1.25 µm and an air concentration of 0.4976 mg ETH50/l using the Multiple-Path Particle Dosimetry model (MPPD V 2.1). The MPPD model is a computational model that can be used for estimating human and rat airway particle dosimetry. The MPPD software is available for download (www.ara.com/products/mppd.htm). The fraction reaching the alveoli was estimated to be 4.92%. The calculation for ETH50 in the acute rat experiment is provided in the attached report (BASF SE 2010a).

In summary, it can be concluded that for micronized ETH50 the inhalation LC50 is greater than the highest technically achievable aerosol concentration level of 4.976 mg/l air (containing 10% active ingredient), or greater than 0.4976 mg ETH50/l air, according to this study's results with MMAD of about 1.2 µm.

B. Severity of the pulmonary effects compared to literature values.

The lung response after the acute 4 hrs exposure, measured by BALF analysis and histopathology indicated an inflammatory response on day 2 post-exposure (pe) that decreased up to 15 days pe, even though the males showed a continued significant increase in the lung weights parameter. Further information on particle clearance from the lungs is not available because tissues were not analyzed for residual ETH50. Microscopic examination did not reveal ongoing lung pathology at 15 days pe.

As requested by the Working Group, we have assembled a short table of information taken from published literature findings of rat lung inflammatory responses to particle exposures

(Table 1). The majority of particle effects on rat lung in vivo are found in studies using intratracheal (IT) installation of the particles and monitoring lung responses after BALF collections and analysis. For another point of comparison we include one study of 3 months' daily inhalation exposures to toner dust (Bellmann et al. 1992). In our inhalation rat in vivo study, we had a number of common endpoints to which comparison across substances and particle types can be made.

In Table 1, a first comparison of inflammatory response, represented by the change in neutrophils (PMNs), shows the ETH50 particle response (71% of total cells) was in a similar range to that seen for IT doses of "Nano-ZnO", "Fine ZnO", and α-Quartz, but half of the response seen after 3-months' exposure to toner dust. Inhalation exposure over 5 days showed a more pronounced inflammatory response to TiO_2, ZnO, and Multi-walled carbon nanotubes (MWCNT) within 2 days after exposures of 50, 12.5, and 2.5 mg/m^3; recovery was seen within 15 days.

In summary, it can be concluded, that the lung inflammatory response seen with ETH50 was in the same range or even weaker than responses seen with other well known particulate materials. Overall, these effects can be considered to be mild in nature and reversible after an adequate period of time after cessation of the treatment. However, the exposure to ETH50 in our study was at maximally achievable aerosol concentration with the test sample amounting to 0.4976 mg ETH50/l (ca. 498 mg/m^3) or about 4.7 - 5.6 mg ETH50/kg bw (using the calculated lung burden of 1.17 mg and the reported body weights of about 250g and 210g for males and females). The achieved lung burden was in the same range as in the studies reported by Landsiedel (2008), Landsiedel et al. (2010) and Sayes et al. (2007).

BASF
The Chemical Company

Table 1. Lung cellular & Tissue responses to ETH50 in Male rats Compared to values for other substances from published literature (Male rats were selected based on available literature; Bellmann et al. used females).

	ETH50 (Tinosorb®A2B) (RCC study 38698, Nov. 2007)	**TiO_2** (Landsiedel et al)[1]	**ZnO** (Landsiedel et al)[1]	**MWCNT** (Landsiedel et al)[1]	**α-Quartz** (Sayes et al. 2007)[2]	**Nano-ZnO** (Sayes et al. 2007)	**Fine ZnO** (Sayes et al. 2007)	**Toner Dust:** carbon black & styrene copolymer (Bellmann et al 1992)[3]
Study description: Exposure	Wistar; 4-hrs, nose-only; MMAD= 1.2 µm; 0.4976 mg ai./l air; Results for Day 2 pe.	Male Wistar rats; nose-only; 6-hr/d, 5 consecutive days; data shown for 2-days pe are **as x fold increase vs. control values**			Crl:CD rats; intratracheal; data at 24 hrs pe			SPF Female rats; nose only, 6h/d, 5d/wk for 3 mos. at 40 mg/m^3 with MMAD 4 µm. Data at end of exposure.
		50 mg/m^3 high dose; MMAD= 0.8 µm	12.5 mg/m^3 high dose; MMAD= 0.9 µm	2.5 mg/m^3 high dose; MMAD= 2 µm	5 mg/kg bw in PBS	5 mg/kg bw in PBS	5 mg/kg bw in PBS	
Lung burden (mg/lung)	1.17 mg	1.636 mg	0.555 mg	0.118 mg	ca 1mg	ca 1 mg	ca 1 mg	3 mg
Particle size (nm)	109 nm (d_{50})	10-50	70-110	10-15 x 100-10,000	1600	90 - 283	90 - 283	NR
Surface area (m^2/g)	43.4	NR	12-24	NR	5.1	12.1	9.6	NR
Total cells (10^6)	62 (7.8 fold increase)	10	9	7	14 (2.3 fold increase)	13 (2.2 fold increase)	10 (1.7 fold increase)	NR
Macrophages (%)	26 (27 % of control)	2	1	0.2	NR	NR	NR	152* fold increase ($p<0.01$)
PMNs (neutrophils; %)	71 (79 fold increase)	500	900	600	60 (32 fold increase)	60 (26 fold increase)	55 (18 fold increase)	141* fold increase ($p<0.01$)
TNFα (pg/ml)	61 (< 22.4 in controls)	NR	NR	NR	NR	NR	NR	NR
Total protein (g/l)	317 (4.7 fold increase)	5	10	5	NR	NR	NR	2.15 * fold increase ($p<0.01$)
Comments:	**Estimated dosage:** 4.7 – 5.6 mg a.i./kg bw	MWCNT=multi-walled carbon nanotubes Dose-related increases and time-related reversals for the parameters shown in this table were seen in the test.			A Quartz PMNs >25% thru 3 mos. pe. NZO & FZO had PMN recruitment (15-20%) at 1-week but reversed at 1 mo. pe. A dose-related increase was reported for the endpoints.			**At 40 mg/m^3 daily exposure 1.1 mg/d; or 4 mg/kg bw/d.** At 10 mg/m^3 changes were not statistically different.

1. Landsiedel, 2008. Short-term inhalation tests of 8 nanomaterials. Presentation Society of Toxicology 47th Annual meeting, Seattle, WA and Landsiedel et al., 2010. Testing Metal-Oxide Nanomaterials for Human Safety. Adv. Mater., 22, 2601-2627 and Landsiedel et al., 2010. Testing Metal-Oxide Nanomaterials for Human Safety. Adv. Mater., 22, 2601-2627
2. Sayes et al 2007. Sayes, C., Reed, K., Warheit, D. Assessing toxicity of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary profiles. Toxicol Sci 97 (1) p 163-180.
3. Bellmann B, et al. 1992. Irreversible pulmonary changes induced in rat lung by dust overload. Environ Health Persp 97, p 189-191.

BASF
The Chemical Company

II Determination of particle/droplet sizes in spray formulations under use conditions

A General - Typical sun spray formulations

Sun spray formulations can be packaged into standard plastic containers and propelled with manual vaporiser (spray) pumps or they can be filled into pressurised containers to be dispensed using propellant gases. The prerequisite for such a spray formulation is to be: either of low viscosity, to be sprayed out of the packaging or to be based on strongly shear-sensitive thickeners, which drastically decrease the viscosity of an emulsion when subjected to shear stress. Sun spray formulations are generally o/w emulsions, based on the same cosmetic components as other sun care emulsions (cream, gel etc). In theory, they can be the same for pump and propellant sprays.

Examples of common ingredients in such o/w formulations are:

- Emulsifiers: Cetyl Phosphate, Potassium Cetyl Phosphate, Lauryl Glucoside, Polyglyceryl-2 Dipolyhydroxystearate, C14-22 Alcohols, C12-20 Alkyl Glucoside
- Emollients: Isopropyl Palmitate, C12-15 Alkyl Benzoate, Trioctanoin
- Moisturizing substances, such as Glycerin

In case of a pressurised sun sprays, the emulsion is mixed with the propellant within the aerosol can. Propellants generally used are Isobutane, Propane, Butane or Dimethyl Ether. The emulsion must be miscible with the propellant and must be stable over time.

B Aspects of the safety assessment of sun spray formulations

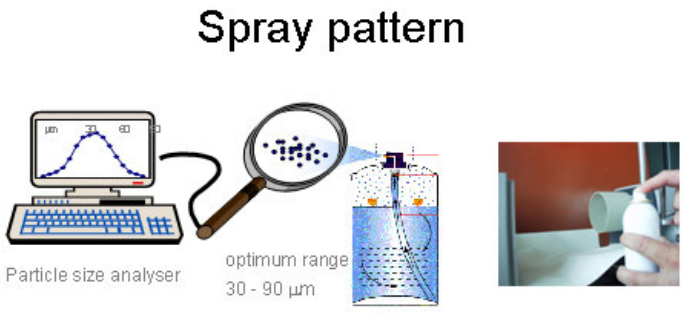
Only cosmetic aerosols comprising very fine droplets/particles lead to a relevant inhalative exposure. As a first approximation, it can be assumed that propellant gas sprays do produce aerosols with inhalable constituents, while this is not expected for the majority of pump-generated sprays, which produce larger droplets. Typically, the mean mass in the primary droplet spectrum of a pump spray is in the range of > 70 µm diameter droplet size, and the portion < 10 µm is clearly below 1%. The size of droplets or particles suspended in the aerosol is decisive for the depth of penetration into the respiratory tract. It is assumed that significant absorption of inhaled substances is to be expected only for the fraction with an aerodynamic diameter of less than 10µm can reach the alveoli. If only the pulmonary available fraction is relevant for exposure (e.g. in case of lung specific effects), the percentage of particles < 10 µm (% < 10 µm) determined in particle size measurements can be considered in estimates of exposure. Of relevance for inhalation exposure is the proportion of the sprayed amount which does not settle on the targeted area sprayed, but instead is carried in the ambient air. According to published literature, this proportion can reach up to 15% in the case of hair spray (Bremmer et al. 2006).

C Droplet/particle size determinations using a Laser Diffraction system (Mastersizer)

The purpose of this investigation was to evaluate the influence of different formulation types and technological parameters such as spray type (propellant or pump spray) or propellant gas concentration on the size and size distribution of droplets in a sunscreen emulsion containing micronized ETH50 as the only UV filter in the formulation.

In general, particle size can be measured with the aid of laser diffraction by aiming a laser beam into the particle cloud. The optimum range of detection is between 30 and 90 µm. Particles present in the aerosol scatter the laser beam, with smaller particles causing higher degrees of scattering than larger ones. The light scattered is measured by photo-detectors positioned at various angles in the detection chamber (see Figure 1).

Figure 1 Mastersizer – Experimental setup



http://www.malvern.de/LabGer/products/iwtm/particle_size.htm

Material and methods:
In our study, two different types of fluid o/w cosmetic emulsions were prepared containing micronized ETH50 (mean diameter 124 nm) at a high concentration of 8 and 10%, respectively (formulation names: GEUV10079-1-2 and GEUV10079-2-2). These emulsions were considered as prototype formulations for ETH50 and were selected based on their technological properties, such as viscosity, stability and spray ability. In addition, a third formulation was prepared as a variation of the second. Only Magnesium aluminium silicate was omitted in this emulsion (GEUV10079-2-3). Details of the formulation compositions can be found in the attached report (BASF SE, 2010b).

These formulations were incorporated in aerosol cans with gas (a blend of propane and butane). The propellant was added at a final concentration of 30 and 40%, respectively. The propellant propane/butane at a concentration of 40% was considered as a worst case scenario with respect to the generation of small droplets based on the experiences of the laboratory (e.g. compared to other gases such as dimethyl ether or nitrogen). The size and the particle size distribution of the droplets were analysed using a Malvern Mastersizer (Malvern, UK). The first and the second formulation were also incorporated into cosmetic pump spray bottles. The type of aerosol valves and spray heads were of typical quality and specification for cosmetic aerosols, e.g. hair sprays (supplier: Precision Company, USA). The particle size was characterized by the mass median diameter ($d_{0.5}$). Other parameters estimated in the experiments were the percentage of droplets with a size below 10 and 1 µm. The measuring distance of droplets was set at 30 cm, because it was considered as a relevant distance under normal use conditions.

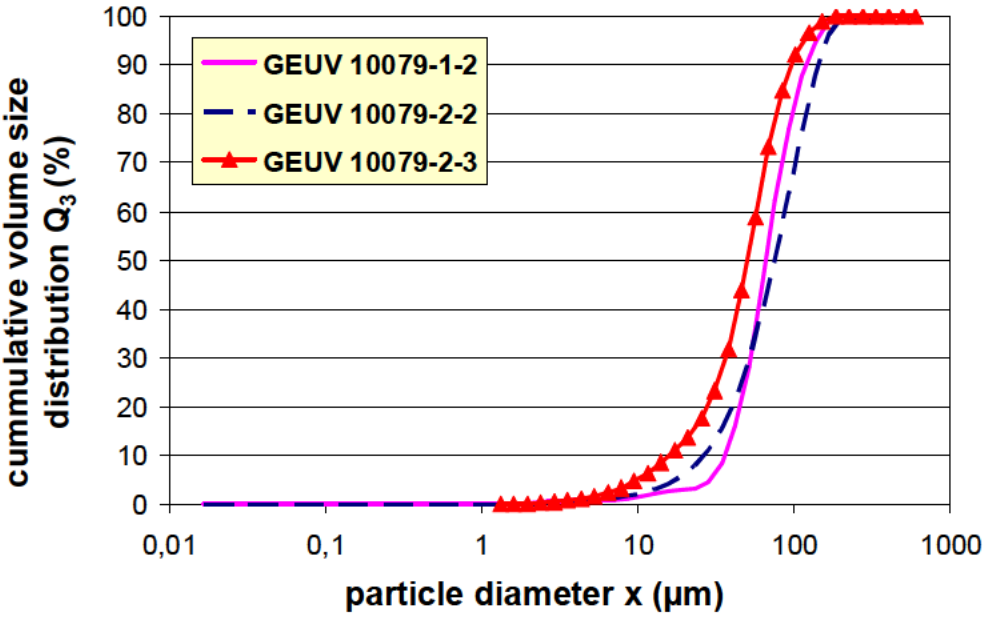
Results:
The results of the spraying experiments with the ETH50 formulations are summarized in table 2 and figure 2.

Influence of the formulation type: It was obvious that formulation 1 produced larger droplets than formulation 2 and 3 (e.g. the $d_{0.1}$ values were about five times higher compared to formulation 2 and 3 for the propellant sprays).
Influence of the propellant concentration: As expected, 40% propane/butane lead to finer aerosols compared to a concentration of 30% propellant.
Influence of the spray type (pump spray versus propellant spray): Interestingly, the pump spray containing formulation 1 produced smaller droplets than the propellant spray with the same formulation. As expected, the pump spray containing formulation 2 generated larger droplets compared to the propellant spray.
For both pump sprays, the droplet fraction below 10 µm was below 1%. For the propellant formulation 2 and 3 this fraction was well below 10%.

Table 2 Droplet sizes for different propellant and pump sprays using laser diffraction
(single measurements for pump sprays and propellant sprays 40%,
duplicates for propellant sprays 30%)

Formulation	o/w Emulsion (GEUV10079-1-2)			o/w Emulsion GEUV10079-2-2			o/w Emulsion GEUV10079-2-3
Conc. Butane/Propane	30%	40%	0% Pump-spray	30%	40%	0% Pump spray	40%
Conc. ETH50 in formulation	8%	8%	8%	10%	10%	10%	10%
Mean Diameter ($D_{0.5}$, volume distribution)	210 / 229 µm	204 µm	132 µm	82 / 78 µm	57 µm	68 µm	50 / 52 µm
Mean Diameter ($D_{0.1}$, volume distribution)	110 / 128 µm	100 µm	53 µm	29 / 29 µm	21 µm	40 µm	16 / 19 µm
Max. fraction of droplets below 10 µm	< 1%	< 1%	< 1%	< 10%	< 10%	< 1%	< 10%

Figure 2 Particle size distribution for 3 different sun screen formulations (8 and 10% ETH50; 40% Propane/Butane) measured by laser diffraction



Conclusion:

The mean droplet size ($d_{0.5}$; volume distribution) for all spray formulations (propellant sprays and pump sprays) using laser diffraction methodology (Malvern Mastersizer) was found to be about 50 µm or above. The droplet fraction below 10 µm was found to be well below 10% (propellant sprays) and 1% (pump sprays), respectively.

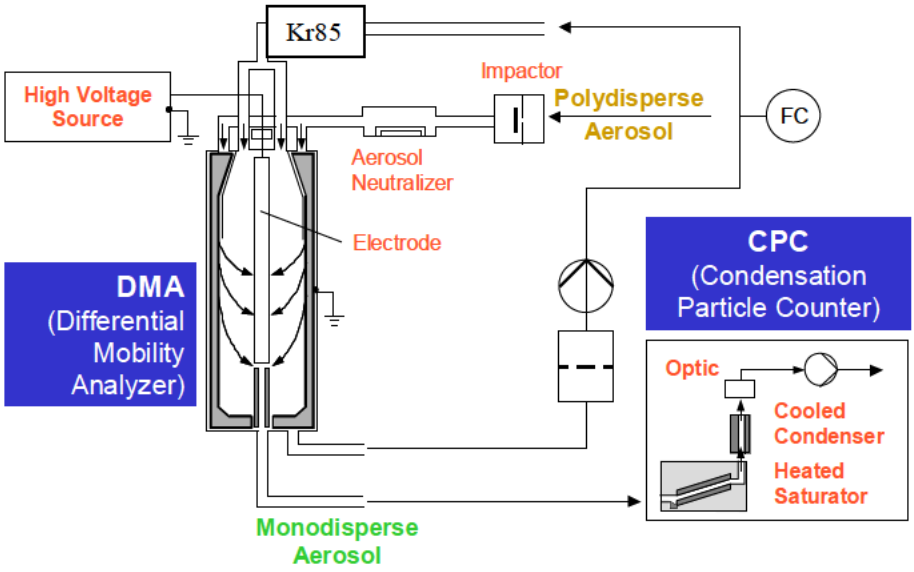
D Droplet/particle size determinations in the submicron range using a SMPS system

The laser diffraction methodology does not allow the detection of tiny particles with a size below 1 µm with certainty. Therefore, the release of aerosols from 3 pressurised ETH50 spray formulations was investigated with a Scanning Mobility Particle Sizer (SMPS) regarding their particle size distribution in the sub-micron range range between 10 and 600 nm (BASF SE 2010c). The atomisation process was operated in an enclosed box to get constant conditions and a particle free background. The setup was similar as described in the recent publication of Hagendorfer et al., 2010. An SMPS equipped with a sample holder for TEM-grids was used in order to sample particle fractions and perform an element analysis by a Transmission Electron Microscope (TEM) and EDX (Energy Dispersive X-Ray)-spectroscopy.

Materials and methods:
The investigated formulations were the same as for the Mastersizer experiments. However, only the 40% propane/butane sprays were tested in the SMPS experiments as they were considered a worst case scenario based on the laser diffraction data.

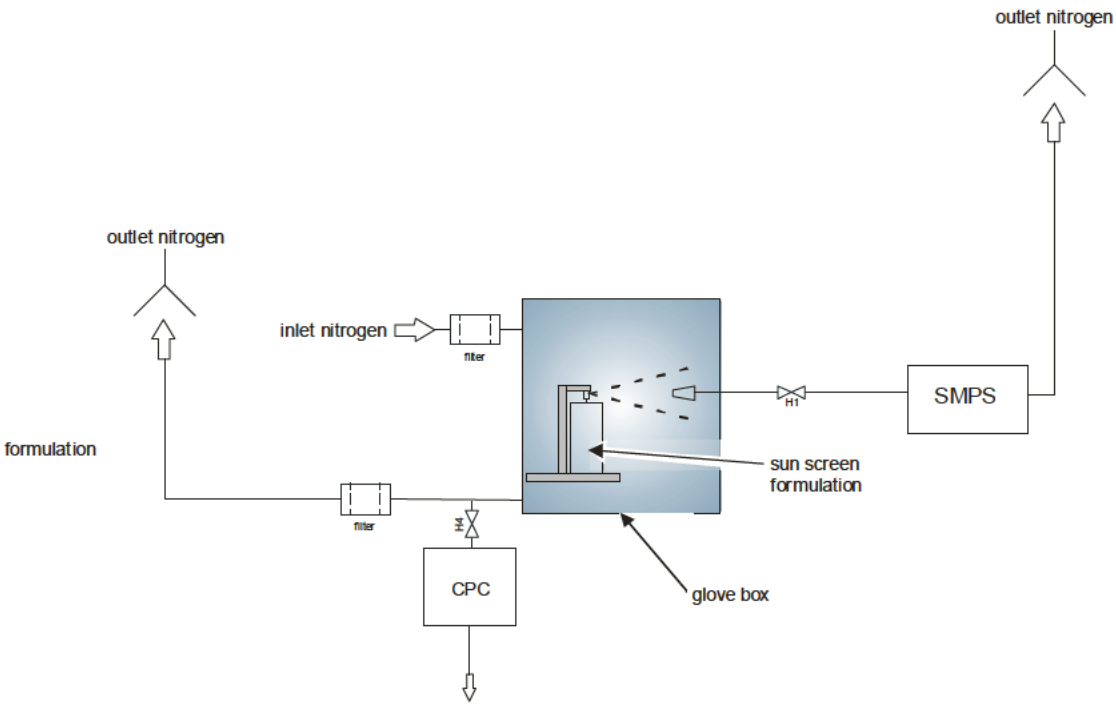
The Scanning Mobility Particle Sizer (SMPS), Figure 3, is based on the principal of the mobility of a charged particle in an electric field. Particles entering the system are charged (using a radioactive source Kr85) such that they have an equilibrium charge distribution. They then enter a Differential Mobility Analyser (DMA) where the aerosol is classified according to electrical mobility, with only particles of a narrow range of mobility exiting through the output slit. This monodisperse distribution then goes to a Condensation Particle Counter (CPC), which determines the particle number concentration at that size.

Figure 3 Principle of a Scanning Mobility Particle Sizer (SMPS)



To avoid an explosive atmosphere, the experiments were done in a glove box (height: 50cm, length: 70cm, width: 70cm) that was inertized with nitrogen. All the spray experiments were carried out under the same experimental conditions, e.g. the spray nozzle was always adjusted at the same height relative to the sampling head (see Figure 4). The same nozzle was always used to ensure comparable conditions for every spray experiment.

Figure 4 SMPS - Glove box and the particle measurement instruments



We used a SMPS for online recording of particle distributions, and a second CPC to monitor the particle concentration in the glove box during the whole measurement time. A closed environment with minimal particle background is needed to perform reliable spray experiments. The closed glove box setup ensured stable conditions and avoided external influences such as air flow. The setup assured a minimal particle background environment with maximum of 10 particles per cm^3 in the measured size range between 10 nm and 600 nm. The particle evacuation after a spray experiment required around 5 minutes of flushing the glove box with nitrogen until reaching acceptable background levels. Before each experiment, the spray cans were shaken 20-times by hand and a 2-minute measurement period with the SMPS followed. The recording time was divided into 1 minute of measurement with spraying followed by 1 minute of measurement without spraying.

Results:

The aerosols generated by the pressurized sun sprays produced a measurable SMPS signal between 10 nm and 600 nm, which was significantly different from the very low background levels of the glove box (see figure 5). However, a differentiation between wet and solid particles by SMPS technology was not possible.

Figure 5 Particle size distributions of three different sun screen formulations obtained with the SMPS

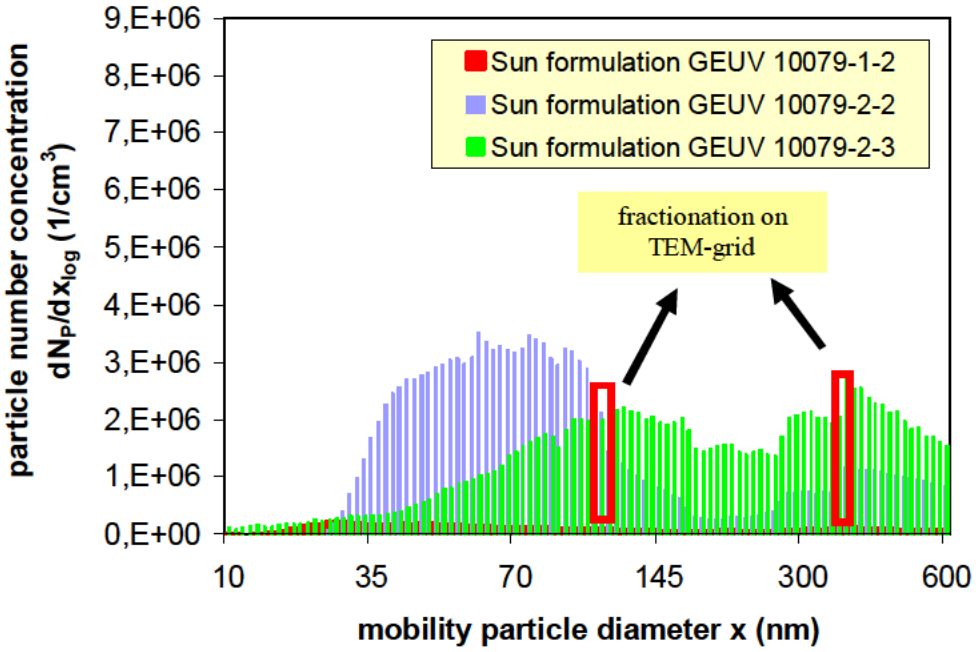


Figure 5 presents the results of the SMPS scans for the three different propellant sun sprays. The first formulation (GEUV10079-1-2) showed particle number concentrations lower than 1000 particles/cm³, which can be considered to be a very low number compared to normal background values (e.g. up to 10,000 – 50,000 in and outside of buildings).

The two other formulations showed particle concentrations above $1 \cdot 10^6$ particle/cm³. These differences might be explained by the different composition of the three formulations. The third sample (GEUV 10079-2-3) was investigated after the second formulation (GEUV 10079-2-2) with the aim to elucidate the increased particle number concentration seen with the second formulation (figure 5, purple bars). It was speculated that the signals detected might have been caused by tiny particles of Magnesium aluminium silicate, which was contained as an ingredient in this formulation. Therefore, the third formulation was prepared without Magnesium aluminium silicate. However, the submicron fraction was still observed with this formulation (figure 5, green bars).

In order to identify the chemical nature of the submicron particles or droplets detected with formulation 2 and 3, two particle size fractions of about 120 nm and 370 nm were then collected on a TEM grid sampler that was connected to the SMPS (see red bars in Figure 5). The TEM-images and EDX-spectra were taken from the third formulation only, as the composition of the second and the third formulation was very similar except the presence of Magnesium aluminium silicate in the second formulation (GEUV 10079-2-2).

The TEM-grids were prepared in deep frozen sample holders (-180°C) to avoid a sublimation or vaporisation of low volatile components under high vacuum. The results showed that no organic particles were present on the TEM-grid. ETH50 was not found in the EDX-spectra. Magnesium aluminium silicate was also not identified. The submicron droplets or particles detected by the

sensitive SMPS have therefore to be of volatile nature whereas ETH50 has a high molecular weight (MW 538) and a very low volatility (calculated: 4.15×10^{-21} Pa at 25°C).

One explanation for the observation of volatile droplets or particles in the submicron range may provide the findings of Chen et al. (1995) who reported that nanoparticles in a range of around 10 nm can be generated through water nucleation in a saturated atmosphere by number concentrations of $c_N \geq 10^5 1/cm^3$ is therefore considered possible. It could be speculated that a coagulation of low volatile nanoparticles (liquid or solid) in the range between 10 nm to 600 nm might have occurred with the o/w sunscreen emulsions, which was then detected by the sensitive SMPS system.

Conclusion:

The SMPS results showed that the presence of submicron ETH50 particles or agglomerates could not be detected in any of the spraying experiments. By EDX and TEM analysis, it could be demonstrated that the submicron fraction, which was observed with one of the formulation types, consisted of volatile molecules or particles. They were most likely formed by water/oil nucleation processes during spraying or either could originate from impurities from the cosmetic raw materials used.

E Particle size distribution of the aerosols in time (not only directly after application)

It is our understanding that during the aerosol's release from the nebulizer and during the travel time to reach the rat's (in case of the inhalation experiment) or the human's breathing zone that the aqueous dispersion could "dry" to some degree by loss of water from the suspension to the air. However, even when water or low volatile compounds leave to some degree the dispersion, the surfactants in the formulation or other ingredients of lower volatility (e.g. moisturizers) would remain with the micronized particles and we assume that this would not change significantly the representative particle size distribution profile.

Further, if the aerosol would dry completely (although this is during use of the product not intended), it is our conclusion that the particles would agglomerate to larger particle sizes rather than disperse as individual small particles of test substance. This conclusion is based on direct observations made when similar suspensions of C-801 (containing ETH50) are placed on grids for SEM and TEM imaging. As shown in the sample image below, the particles are showing the tendency to agglomerate under the intense conditions of the electron microscopy. As seen on the scale bar of the micrograph, the agglomerations are in the microns size range and are not expected to release smaller particles of respirable sizes. More images have been submitted in the Appendix I of Supplement III (August 2008).

Characterization of the Sample [14C] FAT 65080 (Tinosorb A2B)

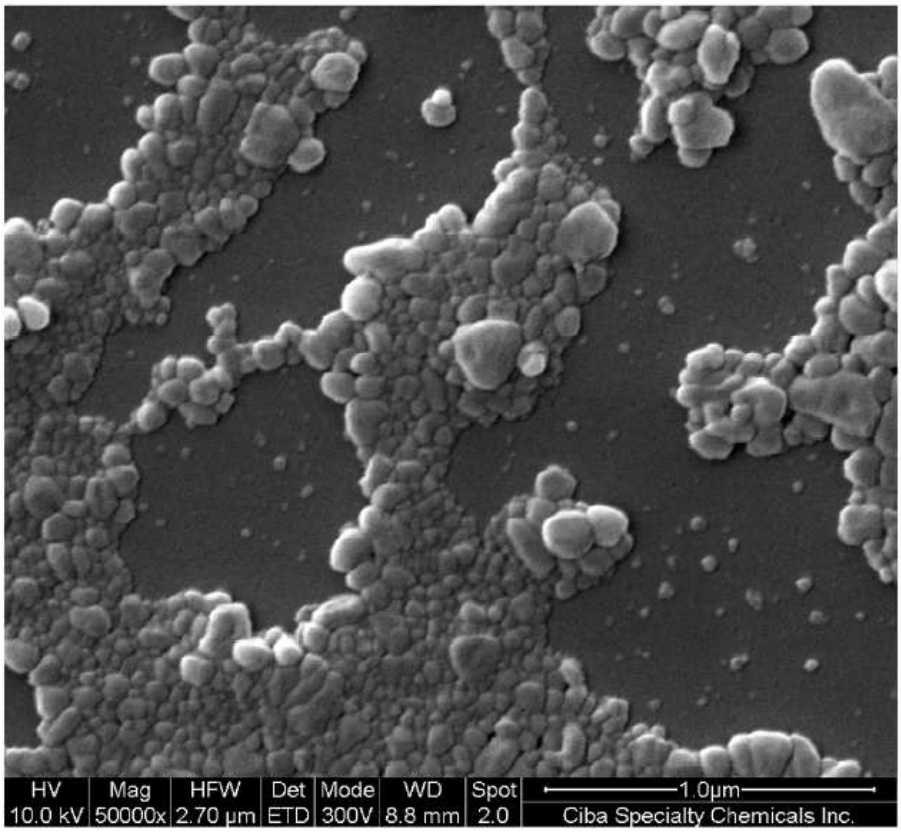


Figure 8: SEM picture of a sample of Tinosorb A2B OP Lot 07612FC7.

III Margin of Exposure Calculations for Inhalation

As it was presented in our Supplement V of 22 April 2010, we take into consideration the potential human exposures from spray-on consumer products; in addition we will take the recommendation from the Nano Expert Working Group meeting of 6 July 2010, and do a comparative exposure assessment of pump spray application vs. the continuous spray applicator that uses compressed gas to deliver the finished sunscreen product to the skin.

Relevance to human exposures via spray-on sunscreen formulations

For our purposes, we will show how the rat inhalation study compares to the estimated human exposure that could result from use of spray-on sunscreen formulations. Regarding the risk assessment, we believe that for insoluble particulate substances the comparison of the lung burden (human exposure vs. animal experiment) is of particular relevance. Therefore, we provide different MoS calculations (related to air concentration, systemic exposure dosage as well as lung burden).

The exposure to respirable particles of ETH50 released by propellant or pump sprays was calculated using the ConsExpo 4.1 model (www.consexpo.com). The currently public available web based

version employs a mathematical module for the determination of inhalative exposures to consumer products. We make the following assumptions which we consider being specific for the application of sun sprays, and which is different from the standard hair spray scenario in the ConsExpo database.

1. Application at 1 mg sunscreen/cm^2 to skin (17,500 cm^2) = 18 g/application
2. ETH50 present in sunscreens at 10% in spray formulation
3. Application of 1800 mg a.i.
4. Mass generation rate of spray: max 1 g/sec (measured value for propellant spray)
5. Spray duration (in total): ca. 0.5 min (estimated from mass generation of spray and application volume to the skin)
6. Exposure to aerosol during application: about 5 minutes (estimated)
7. Weight fraction non-volatile in the formulation: 0.1 (i.e. 10% ETH50)
8. Estimated median particle size $d_{0.5}$ (volume distribution; based on Mastersizer data):
   - Pump spray: 60 µm (worst case)
   - Propellant spray: 50 µm (worst case)
9. Variation coefficient: 0.3 (estimated from Mastersizer distribution curves)
10. Droplet distribution: a normal distribution of data was assumed
11. Inhalation cut-off diameter: 15 µm (default)
12. Airborne fraction (estimations based on default values suggested by Bremmer et al., 2006)
    - Pump spray: 20% (according to Bremmer et al., trigger sprays, mean particle size ≥ 50 µm)
    - Propellant spray: 50% (according to Bremmer et al.; can sprays, mean particle size ≥ 50 µm)
13. Human ventilation rate: 33 m^3 per day (light exercise), ca. 1.375 m^3 per hour

For all other parameters, the default use data for hairspray are used.

Using the above described assumptions and given default values of the model, the following results presented in table 3 were obtained (the details of the scenarios and the results can be found in the attached reports BASF SE, 2010d and BASF SE, 2010e).

Table 3 ConsExpo estimations for pump and propellant sun sprays containing 10% ETH50 in a sunscreen formulation (see BASF 2010d and 2010e))

Exposure	Pump Spray	Propellant Spray
Mean event concentration (5 minutes)	0.32 mg/m^3	1.26 mg/m^3
Acute internal dose by inhalation	0.000607 mg/kg bw	0.00238 mg/kg bw

A Margin of Exposure can now be calculated by comparing the exposure data obtained in the animal experiment during 4 hours to a mean event concentration or to the acute internal dose by inhalation.

Table 4 Margin of Exposure calculation using the mean event concentrations and the acute internal doses

Exposure rat (4h)	**Pump Spray** Mean event concentration	**Propellant Spray** Mean event concentration	**Pump Spray** MoE (Exposure rat/mean event conc.)	**Propellant Spray** MoE (Exposure rat/mean event conc.)
0.4976 mg/l (497.6 mg/m^3)	0.32 mg/m^3	1.26 mg/m^3	1555	395
Acute internal dose (rat)	**Acute internal dose (human)**	**Acute internal dose (human)**	**Pump Spray** MoE	**Propellant Spray** MoE
4.7 - 5.6 mg/kg bw	0.000607 mg/kg bw	0.00238 mg/kg bw	7743 - 9225	1975 - 2353

The MoE obtained above by comparing exposure in the acute animal experiment during 4 hours without mortality and only mild signs of lung inflammation to a mean event exposure of only 5 minutes is considered to be a worst case scenario and maybe even higher if Haber's rule is applied.

The Magnitude of the MoE (1975 - 2353) for the propellant spray which was derived from the systemic exposure doses (acute internal doses in rat und human) does not indicate that systemic toxicity might be relevant under practical use conditions.

With respect to a risk assessment, we think that for insoluble particles the lung burden after potential inhalative exposure is of particular relevance. In the following, MoE calculations are presented by comparing the lung burdens in the acute inhalation study with the potential lung burden of the consumer in the above scenarios for pump and propellant sprays.

Table 5 Calculation of Margin of Exposure by comparing the lung burdens in rat and human

Parameter	**Pump spray**	**Propellant spray**
Mean event concentration (5min)	0.32 mg/m^3	1.26 mg/m^3
Human ventilation rate	33 m^3/d = 1.375 m^3/h	33 m^3/d = 1.375 m^3/h
Exposure time consumer	5 min	5 min
Inhaled alveolar amount	0.32 mg/m^3 x 5 min/60 min x 1.375 m^3/h = 0.0367 mg	1.26 mg/m^3 x 5 min/60 min x 1.375 m^3/h = 0.144 mg
Human lung weight	ca. 1 kg	ca. 1 kg
Human lung burden (per g lung)	0.0367 mg/kg lung = 0.0367 µg/g	0.144 mg/kg lung = 0.144 µg/g
Rat lung burden (per g lung) (see also chapter 1 A)	1.17 mg/g	1.17 mg/g
Margin of Exposure (lung burden rat / lung burden human)	1.17 mg/g / 0.0367 µg/g **= 31 880**	1.17 mg/g / 0.144 µg/g **= 8 125**

Conclusion:

The Magnitude of the MoE values obtained in the presented user scenarios by comparing the modeled air concentrations or the internal doses or the lung burdens after the potential inhalation of ETH50 do not indicate a risk for the consumer. In particular, a comparison of the lung burden gave a MoE of 31880 for pump sprays and 8125 for propellant sprays. We conclude that sun sprays containing ETH50 at a concentration of up to 10% can be safely used and no adverse effects are to be expected. This assessment is valid for pump sprays as well as for propellant sprays.

IV Margin of Safety Calculation for Dermal Exposure to ETH50

This section is modified from that given in our Supplement No. IV. Given the particulate nature of the UV Filter ETH50 and the inclusion of nanometre sized particles (diameter <100 nm) in the marketed product, we consider the safety characterization should include both particle and soluble chemical exposures from topical application of cosmetic products. The collective non-clinical study results did not show any new toxicity effects from micronized ETH50 when compared to not micronized ETH50. Systemic concentrations of ETH50 in plasma did not show a clear dose or time relationship by oral gavage or dermal dosing.

Throughout this Addendum we presented animal dosages as mass of ETH50. As a relative perspective on human skin exposures to particles of micronized ETH50, we use a 10% ETH50 (a.i.) concentration applied in sunscreen at 1 mg/cm^2 to 1.75 m^2 surface area of a 60 kg body weight

human. We did not see any new or different types of adverse effects with micronized ETH50; in fact, the in vitro dermal absorption rate of ETH50 as a worst case particle size of about 80 nm on normal skin was about 5-fold lower than that from larger-sized (6 µm) micronized ETH50 (RCC 2005b and RCC 2007b). Even application to damaged skin in vitro did not give a meaningful increase in absorption of ETH50 compared to normal skin (results see also Addendum IV). We consider ETH50 to be of very low biological availability after dermal or oral exposures.

Margin of Safety. While we consider ETH50 not to be absorbed across human skin or absorbed systemically to a relevant extent, the calculation of a Margin of Safety is provided below. Taking the standardized approach to risk characterization, we compare systemic estimated human exposures that could be assumed to result from 10% ETH50 in cosmetic product applied as an 80nm particle and as a 6µm particle to normal skin. These data are then compared with the Systemic Exposure Dosage (SED) estimated from the subchronic dermal study in rats (CIT 2008a) using the in vivo NOAEL and the dermal absorption rate found in the in vitro penetration experiments with rat skin (RCC 2007b). The estimated Margin of Safety calculations for micronized and non-micronized ETH50 applied to normal skin using % absorption data are presented in Table 6.

Table 6 Dermal exposure - MoS calculations for ETH50 using percentage absorption of the amount applied

Parameter	**Comparative MoS calculations for ETH50** Based on human skin in vitro study results for micronized (80 nm) and non-micronized particle size (6 µm) on normal skin	
	Micronized ETH50	**Non-Micronized ETH50**
Adult Body weight	60 kg	60 kg
Body surface area	17.500 cm^2	17.500 cm^2
Sunscreen applied (if at 1 mg/cm^2)	18 g	18 g
ETH50 applied (10%)	1800 mg	1800 mg
Skin absorption (human) RCC B23624 April '07 RCC A00112 August '05	0.06% of applied dose	0.28% of applied dose
Systemic Exposure Dose (human)	0.018 mg/kg bw/day	0.084 mg/kg bw/day
NOAEL Rat 13-wk dermal study (CIT 32404 TCR, Aug' 08)	1000 mg/kg bw/day	1000 mg/kg bw/day
Skin absorption (rat) (RCC B23624 April '07)	1.38% of applied dose	1.38% of applied dose
Systemic Exposure Dose (rat)	13.8 mg/kg bw/day	13.8 mg/kg bw/day
MoS (SED Rat / SED Human)	**766**	**164**

Margin of Safety calculations for ETH50 using dermal absorption reported in µg/cm^2:

Using the absorption rate as reported in µg/cm^2, the MoS can be calculated from the external doses and the respective dermal absorption rates with the following equation:

$$\text{Margin of Safety} = \frac{\text{NOAEL rat, dermal (mg/kg bw) x Skin absorption, rat (µg/cm}^2\text{)}}{\text{Human external dose (mg/kg bw) x Skin absorption, human (µg/cm}^2\text{)}}$$

- NOAEL rat, 13 weeks, dermal: 1000 mg/kg bw
  CIT 32404 TCR, Aug' 08
- Skin absorption, rat: 27.2 µg/cm^2
  micronized ETH50, RCC B23624 April '07
- Human external dose: 1800 mg a.i./ 60 kg bw = 30 mg/kg bw
- Skin absorption human: 1.2 µg/cm^2
  micronized ETH50, RCC B23624 April '07
- Skin absorption human: 5.4 µg/cm^2
  non-micronized ETH50, RCC A00112 Aug '05

MoS for micronized ETH50:

$$\text{MoS} = \frac{1000 \times 27.2}{30 \times 1.2} = \mathbf{756}$$

MoS for non-micronized ETH50:

$$\text{MoS} = \frac{1000 \times 27.2}{30 \times 5.4} = \mathbf{168}$$

These comparative calculations each show ETH50 exceeds the accepted MoS of 100 for human exposure to UV filters and that it can be considered safe for topical use in humans at concentrations of 10% in a sunscreen applied to human skin. A calculation of the MoS was not considered as we do not think that a whole body exposure scenario using data from damaged human cadaver skin is meaningful. However, it can be expected that confined areas of potentially higher skin permeability (e.g. regions of diseased or damaged skin) would not change the outcome of our calculations significantly as the absorption of ETH50 on damaged skin was still found to be below 1% of applied dose (under worst case conditions using tape stripped cadaver skin).

Although the MoS values of 164 and 168 for non-micronized ETH50 are considerably lower than for the micronized substance, this is considered to be a conservative scenario. The starting point for the systemic exposure calculation in the rat, the 13-week dermal NOAEL, was obtained at the highest tested dose level of 1000 mg/kg bw/day, which is the upper limit for oral and dermal doses according to current OECD test guidelines for repeated dose toxicity studies in rodents. In the dermal repeated dose study with ETH50, no signs of systemic toxicity (haematology, blood biochemistry, macroscopy and histopathology) were observed at this limit dose of 1000 mg/kg bw/day. The plasma levels of ETH50 ranged at the end of the 13-week exposure period between 2.4

ng/ml and 11.8 ng/ml in males and females, respectively. It is expected that due to the limited dermal absorption of the substance (only 1.38 % in vitro on rat skin) and the corresponding low bioavailability the actual NOAEL would be considerably higher if larger doses were tested.

This assumption is supported by a bone marrow micronucleus test (MNT) with duplicate intraperitoneal applications of up to 1000 mg micronized ETH50/kg bw/d (CIT 2008). Although we are aware that a duplicate administration cannot directly be compared with repeated doses over 3 months, the high ETH50 plasma levels in the MNT were not associated with relevant toxicity. This large dose with systemic availability induced only minor signs of toxicity (piloerection and hypoactivity), which was also observed in a reference item in a parallel group, and these signs are therefore not correlated with the systemic exposure to the substance. Plasma concentrations ranged between 3,300 and 7,200 ng ETH50/ml in males and females, respectively 24 hours after the second treatment (see page 19 of the report). These concentrations are a factor of 280 - 2900 higher than the plasma concentrations measured in the subchronic dermal study in rats (2.47 and 11.81 ng/ml after week 13, see table 13 of this document). From these findings it can be assumed that ETH50 would not lead to relevant systemic toxicity even above the limit dose of 1000 mg/kg bw due its low bioavailability.

V Potential for tissue accumulation of ETH50.

The partition coefficient log $K_{o/w}$ was estimated to be > 5.6 based on the individual solubilities in n-octanol and in water (RCC 2005a). Using a model calculation, the log $K_{o/w}$ was calculated to be 10.4.
Due to the log $K_{o/w}$ > 5.6, a concern over potential accumulation of ETH50 in the body was expressed.

This section is slightly modified from that given in our Supplement V. In addressing this concern we present another summary of the previously submitted studies, beginning with tissue recoveries in the AD(M)E oral and dermal studies and then the test substance concentrations in blood/plasma measured during a 13-week dermal study. Furthermore, we are providing arguments why a tissue accumulation for substances with a very high log $K_{o/w}$ and a low water and fat solubility is not to be expected.

Oral AD(M)E. In one in vivo oral ADE study in rats dosing was with micronized ETH50 (d_{50} ~87 nm) by single oral gavage of 100 mg a.i./kg bw (RCC 2007c). A total of 94% of applied radioactivity was recovered during the study; 94% of dose (99.8% of radioactivity) was found in feces and 0.06% of dose in urine. The blood AUC was 17.9 µg.h/g with an elimination half-time of 31 hours; at 96-hours after dosing the blood and plasma radioactivity was at or below the LOQ of 0.03 µg-equivalents ETH50.

Table 7. Elimination time points for urine & faeces after oral gavage with ^{14}C-ETH50 as (d50) 87 nm particles.

(RCC B23613)	**Excretion**	
Urine	Time period	**[% of dose]**
	0 – 24 h	**0.04**
	24 – 48 h	**0.01**
	48 – 72 h	**<0.01**
	72 – 96 h	**<0.01**
	Subtotal	**0.06**
Feces		
	0 - 24 h	**92.27**
	24 - 48 h	**1.06**
	48 - 72 h	**<0.01**
	72 - 96 h	**<0.01**
	Subtotal	**93.34**
Cage Wash		**0.10**
Total Excretion		**93.49**

Within 48-hours quantifiable ETH50 was not found in urine and feces; the collection times and recoveries are shown in the Table 7.

In the tissues for each animal dosed, all tissues, including fat, did not contain quantifiable ETH50 (all values below LOQ); only the remaining carcass showed ETH50 above LOQ. The results are shown in Table 8; it should be noted that carcass represented only about 0.07% of applied dose.

Table 8. Residual radioactivity in selected tissues and organs of male rats 96 hours after single oral administration of [^{14}C]FAT 65080 (ETH 50) at a nominal dose level of 100 mg/kg body weight

Residues 96 hours after administration [µg equivalents per g]							
Animal No.	1	2	3	4	**Mean**	SD	LOQ[a]
Dose [mg/kg]	115.9	113.7	116.6	115.9	**115.5**	1.2	
Blood	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**	0.003	0.091
Plasma	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**	0.004	0.091
Liver	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**	0.004	0.091
Kidneys	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**	0.004	0.091
Fat	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**	0.018	0.182
Muscle	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**	0.005	0.091
Carcass	0.097	0.077	0.071	0.066	**0.078**	0.014	0.012

Residues 96 hours after administration [percent of dose]							
Blood	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Liver	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Kidneys	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Fat	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Muscle	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Total Tissues[b]	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Carcass	0.08	0.07	0.06	0.06	**0.07**	0.01	
Total Residues	**0.09**	**0.08**	**0.06**	**0.06**	**0.07**	0.01	

a) LOQ = Limit of Quantification; **b)** Residues found in the excised part of the tissues and organs

In a second oral AD(M)E in vivo study in rats that used ETH50 at particle size 6 µm (d_{50}) dosed once by oral gavage of 100 mg a.i./kg bw/d, a total of 98% of applied ^{14}C-ETH50 was recovered during the study (RCC 2007d). Blood and plasma AUC were 65 and 114 µg.h/g, respectively over 96 hours; quantifiable radioactivity was still apparent at the 96-hour time point and, estimated over 48-hours, had a 13-hours estimated half-time for elimination. Feces accounted for 97% of the dose (99.2% of recovered radioactivity) within 48-hours; urine showed only 0.71% of the dose for total excretion of 98% of dose. The excretion results are shown in Table 9.

Table 9. Elimination time points for urine & feces after oral gavage with ^{14}C-ETH50 as (d50) 6µm particles.

[RCC A89280]		**Excretion [% of dose]**
Urine	0 - 24 h	**0.53**
	24 - 48 h	**0.12**
	48 - 96 h	**0.06**
	Subtotal	**0.71**
Feces	0 - 24 h	**94.74**
	24 - 48 h	**2.44**
	24 - 48 h	**0.04**
	Subtotal	**97.22**
Cage Wash		**0.02**
Total Excretion		**97.95**

Unlike the results for the fully micronized ETH50 (87 nm), the larger particle size ETH50 (6 µm) mixed with 14C-ETH50 showed a different pattern of distribution of the radioactivity in tissues and organs (Table 10). However, examination of the % of dose values shows only fat with 0.01% of dose and carcass with 0.25% of dose could be reliably quantified.

Table 10. Residual radioactivity in selected tissues and organs of male rats 96 hours after single oral administration of [^{14}C]- ETH 50

Residues 96 hours after administration [µg equivalents per g]							
Animal No.	1	2	3	4	**Mean**	SD	LOQ[a]
Dose [mg/kg]	101.8	108.0	102.0	103.1	**103.7**	2.9	
Blood	0.069	0.070	0.058	0.055	**0.063**	0.008	0.022
Plasma	0.114	0.106	0.089	0.088	**0.099**	0.013	0.021
Liver	0.119	0.097	0.101	0.120	**0.109**	0.012	0.022
Kidneys	0.089	0.075	0.093	0.096	**0.088**	0.009	0.023
Fat	1.761	3.023	1.262	0.801	**1.712**	0.958	0.022
Muscle	0.037	0.036	0.034	0.045	**0.038**	0.005	0.022
Carcass	0.214	0.326	0.245	0.220	**0.251**	0.051	0.012

Residues 96 hours after administration [percent of dose]							
Blood	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Liver	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Kidneys	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Fat	0.02	0.01	0.01	<0.01	**0.01**	<0.01	
Muscle	<0.01	<0.01	<0.01	<0.01	**<0.01**	<0.01	
Total Tissues[b]	0.02	0.02	0.02	0.02	**0.02**	<0.01	
Carcass	0.21	0.34	0.24	0.22	**0.25**	0.06	
Total Residues	**0.23**	**0.36**	**0.26**	**0.23**	**0.27**	0.06	

a) LOQ = Limit of Quantification
b) Residues found in the excised part of the tissues and organs

Dermal rat in vivo ADE.

In our 2005 dossier, we included results from a rat in vivo dermal absorption study in which ^{14}C-ETH50 was mixed with ETH50 and milled to d_{50} 440 nm; the aqueous suspension was applied for 6-hours to shaved area of rat skin at 2 mg a.i./cm^2 in a single application (RCC 2005c). Recovery was 93-97% of total applied radioactivity across the 72-hours' study duration.

About 91% of the applied topical dose could be removed from the dose site. As shown in Table 11, the systemically absorbed radioactivity was about 0.15% of applied dose up to 72-hours. Whole blood contained less than 0.1% of dose at each time point; the highest concentration occurred between 6 and 24 hours after dosing reaching 0.2 µg-equivalents in plasma. At 48- and 72- hours after dosing the concentrations were below the limit of quantification.

Tissue residues considered low; unfortunately, fat was not measured separately but would be included as part of the Remaining Carcass value: up to 0.07 % of dose. This value is in the same range as seen in the 2 oral ADE studies reported above; these oral studies also show about 0.01% of dose in fat, and in view of the similar systemic absorption values, it can be concluded that after dermal application, fat residues would not be expected to be significantly different.

Summary of AD(M)E studies. In comparing the endpoints of oral versus dermal dosing with 14C-ETH50, it becomes apparent that the oral and dermal studies with the smaller sized particles (87nm to 404 nm, d_{50}) are consistent in the several endpoints, whereas the oral study with the 6-µm sized particles showed higher amounts of 14C-ETH50, as % of dose, for tissue residues, systemic uptake, and elimination time.

Table 11
Excretion & Residues [percent of dose] after Dermal Application of ^{14}C-ETH50

[RCC Study A22432] Dose Level	1829 µg/cm²			
Sacrifice Time Point	(6 h)	(24 h)	(48 h)	(72 h)
Urine				
0 - 6 h	0.01	0.01	< 0.01	< 0.01
6 - 24 h	-	0.03	0.02	0.02
24 - 48 h	-	-	0.01	0.01
48 - 72 h	-	-	-	< 0.01
Subtotal	0.01	0.04	0.04	0.05
Feces				
0 - 6 h	< 0.01	< 0.01	< 0.01	< 0.01
6 - 24 h	-	0.02	0.02	0.01
24 - 48 h	-	-	< 0.01	< 0.01
48 - 72 h	-	-	-	< 0.01
Subtotal	< 0.01	0.02	0.03	0.03
Cage Wash	< 0.01	< 0.01	< 0.01	< 0.01
Total Excretion	**0.02**	**0.06**	**0.07**	**0.08**
Residues				
Whole Blood*	< 0.01	< 0.01	< 0.01	< 0.01
Skin Non-Treated Area*	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal Tract	0.03	0.01	< 0.01	< 0.01
Liver and Kidneys	0.01	< 0.01	< 0.01	< 0.01
Remaining Carcass	0.05	0.04	0.04	0.07
Subtotal	0.09	0.06	0.04	0.07
Systemic Absorption	**0.11**	**0.12**	**0.11**	**0.15**

* Residues determined in the taken part of the specimen

The dermal and oral administration showed only low absorption amounts (0.01 to 0.7% of dose) and elimination curves indicating the substance is cleared from blood and tissues; therefore, it is our interpretation that ETH50 should not be expected to accumulate in tissues.

Dermal 13-Week rat study.

The rat 13-week dermal dosing study used ETH50 micronized to (d_{50}) 109 nm particle size (CIT 2008a). ETH50 was applied at dosages of 150, 500, and 1000 mg/kg bw/d. Topical daily dosing was as micronized ETH50 suspended in a cream-like vehicle; dose sites for all animals were rinsed each day about 6-hours after dose applications.

The test substance concentrations were determined in plasma samples taken after 8 days and 13 weeks in all groups except the untreated control group and at 15 weeks in treatment-free group animals. The plasma analytical results are shown in Table 12.

Table 12. Plasma ETH50 Concentrations (ng/ml)[1] in 13-Week Dermal Rat Study (CIT 2008b)

CIT No. 32404 TCR	Male					Female				
Group Number	2	3	4	5	6	2	3	4	5	6
Dosage (mg a.i./kg/day)	0	150	500	1000 High-dose I	1000 High-dose II	0	150	500	1000 High-dose I	1000 High-dose II
Day 8	0	0.79	1.36	4.17	1.21	0	0.36	5.62	3.04	1.39
Week 13	0	2.39	3.48	2.36	2.47	0	2.05	9.82	2.10	11.81
Week 15 (after reversibility)	*n.a*	*n.a*	*n.a*	1.98	0.39[∇]	*n.a*	*n.a*	*n.a*	0.39	<0.8[#]

1. LOQ = 0.8 ng/ml
[∇]: mean concentration with exclusion of the N26873 value (16.8 ng/mL), considered to be aberrant
n.a: not applicable.
[#]: missing values for animals N27054 and N27055

As stated in our Supplement III document, while measurable ETH50 is reported in the samples collected, the plasma concentrations do not show consistent dose-related or duration-of-exposure related increases. In males and females of the low- and mid-dose groups, the increase in plasma concentrations could suggest a dose-related trend, but at highest doses, the trend does not hold. Comparison of males in high dose group I (without restrictive collar) to high dose group II (with restrictive collar) suggests that oral ingestion of test item could be responsible for the observed blood concentrations. However, in females of the high dose groups, this tendency does not appear.

Assessment of ETH50 Accumulation in tissues.

As discussed in the ADE studies, dermal and oral dosing to rats does result in systemically measurable ^{14}C-ETH50. Accordingly, the plasma concentrations seen in the 13-weeks dermal study are not unexpected. A comparison of the two studies shows the following:

> In the dermal ADE rat study, 6-hours after dosing with about 2 mg/cm^2 to about 10 cm^2 skin (20 mg/ 0.2 kg bw ~=100 mg/kg bw), plasma reached about 0.2 µg-equivalents ETH50/g (~200 ng/ml plasma) and then by 48-hours was not quantifiable. Tissue residues were lower, fat is estimated to be about 2 ng ETH50/g tissue, and followed similar elimination patterns as plasma.
>
> In the 13-week dermal study, ca. 20 ng ETH50/ml plasma was the highest measured plasma concentration during the study (CIT report 2008b, page 1007-1008). This is the highest result even with daily applications of 10-times higher topical dosages given 91 times longer in this 13-week study.

In the AD(M)E studies and in the 13-week dermal study the plasma levels showed a clear elimination pattern. Even if fat or tissues samples were not analyzed for ETH50 residues at the end of this study, the metabolic and elimination patterns discussed above suggest that if ETH50 were present in the fat, it would move from tissue to plasma and be eliminated. The lack of sustained levels of ETH50 in plasma after dosing is stopped further supports our opinion that ETH50 is not

accumulated in fat or other tissues, even after 13-weeks of exceedingly high dosages, because systemic ETH50 moving from tissues to plasma is not apparent.

In addition, to the above presented toxicokinetic data, the physico-chemical properties can be evaluated with respect to a concern for accumulation. It is known that certain properties of a substance indicate that an accumulation in an organism is not likely. For example, in the REACH Guidance document "Guidance on information requirements and chemical safety assessment. ChapterR.11: PBT Assessment, May 2008" (ECHA 2008) indicators for a limited bioconcentration of a substance in the environment are provided. Used within a weight of evidence approach and with expert judgment a chemical may be considered as not bioaccumulative, if e.g. an octanol-water partition coefficient log $K_{o/w}$ is > 10. It is furthermore mentioned in the document that - although the scientific database has limitations with regard to such an assessment - a calculated log $K_{o/w}$ of 10 or above can be taken as an indicator for showing reduced environmental bioconcentration.

In case of ETH50, a log $K_{o/w}$ of > 5.6 was calculated using the obtained solubility data in n-octanol and water. A model calculation provided a log $K_{o/w}$ of 10.4.
Furthermore, we expect that the very low solubility of ETH50 in lipophilic media (as a surrogate for fat tissue) makes a relevant accumulation potential unlikely. For example, solubility values of 0.02%, 0.01, or 0.003% were measured for ETH50 in Caprylic/Capric Triglyceride, Jojoba oil and Mineral oil, respectively (see Dossier, page 8, 08 Nov. 2005). This expectation is also supported by experimental data as only very low levels of ETH50 after acute oral application were found in fat tissue of rodents.

In summary, we conclude that both observations, a high log $K_{o/w}$ (calculated value >10) and the low lipid solubility, can be taken as additional indicators for a limited bioaccumulation potential.

VI. CONCLUSIONS: Overall relevance to human exposures and safety assessment

In our earlier submissions supporting ETH50 as safe for use as UV Filter in cosmetic products, we have consistently shown Margin of Exposure values far above the MoS = 100. In light of the discussion presented above in this document, we would further point out that our MoS calculations are exceedingly conservative and in some instances have used even worst-case type estimations.

We have extended the previous safety assessments to also include spray-on cosmetic products because the spray aerosol is not expected to contain significant amounts of respirable sized components and any inhaled spray would be adsorbed in the nasopharyngeal region with out adverse local or systemic effects. This is supported by exposure data generated with spray formulations which indicate that the fraction of particles or droplets that could reach the lungs is well below 1% and 10% for pump and propellant sprays, respectively.

We offer the following points substantiating the additional factors rendering the rat data as conservative over estimations of the human exposure situation.

1. In our in vitro percutaneous penetration study we showed rat skin penetration (1.38% of dose) was about 23-times higher than excised human skin (0.06 % of dose).

2. Even on damaged human cadaver skin the in vitro percutaneous penetration rate remains less than 1% of applied ETH50.

3. A number of the absorption estimates are, in some cases, based on analytical values below the Limit of Quantification.

4. In our repeated dosing studies with rats, oral and dermal NOAELs were 1000 mg/kg bw/day which is the upper limit dose for regulatory repeated dose studies.

5. The observed lung findings after acute inhalation for 4 hrs and subsequent broncho-alveolar lavage of maximal technically achievable aerosol concentration of ETH50 showed similar or even weaker responses compared to other well-known insoluble particulate substances.

6. Investigations of aerosols that were generated by spraying ETH50 containing propellant and pump sprays showed that the fraction of very small particles/droplets is low. The release of ETH50 submicron particles was not detected by TEM and EDX analysis. A conservative exposure scenario does not indicate a health risk when data from an acute inhalation experiment with broncho-alveolar lavage is taken into account. Thus, we believe that any impacts of even the smallest sized particles in ETH50, including spray-on applications, have been addressed adequately in our studies and safety assessment.

7. A relevant accumulation in the human body is not to be expected based on the physicochemical properties (high log $K_{o/w}$ and very low solubility in fat tissue) as well as the available toxicokinetic data.

Based on the toxicology test results and Margins of Safety for dermal applications, it is concluded that micronized ETH50, should be considered safe for use at concentrations of at least 10% in sunscreens and cosmetics products. Regarding spray applications, aerosol measurements with pressurized and pump sprays indicate that the potential inhalative exposure of the consumer is very low and considered to be safe.

VII References

BASF SE, 2010a.Calculation of lung burden of ETH50 in the 4-hour acute inhalation toxicity study in rats with FAT 65'080 (RCC report, B38698) using Multiple-Path Particle Dosimetry Model (MPPD v 2.1)

BASF SE, 2010b.Droplet sizing of sun sprays containing a new cosmetic UV filter (ETH50) using a Laser Diffraction system (Malvern Mastersizer)

BASF SE, 2010c. Measurement of the droplet size distribution of sun screen formulations containing ETH50, report date 12 Dec 2010

BASF SE, 2010d. ConsExpo 4.1 report, ETH50 Pump Spray (10% ETH50), report date 19 Dec 2010

BASF SE, 2010e. ConsExpo 4.1 report, ETH50 Propellant Spray (10% ETH50), report date 18 Dec 2010

Bellmann et al, 1992. Irreversible pulmonary changes induced in rat lung by dust overload. Env. Health Perspectives, 97, 89-191

Bremmer et al, 2006. Cosmetic Fact Sheet To assess the risks for the consumer, updated version for ConsExpo 4, RIVM report 320104001, page 18 pp, available from the following internet site: http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf

Chen et al, 1995. Electro spraying of conducting liquids for monodisperse aerosol generation in the 4 nm to 1.8 µm diameter range. J Aerosol Sci., 26, 963-977

CIT, 2008a. 13-week toxicity study by cutaneous route in rats followed by a 2-week treatment-free period. CIT study no. 32404 TCR. 1 August 2008

CIT, 2008b. Rat plasma concentration for individual animals in 13-week dermal dosing study; CIT 32404 TCR, page 44 and pages 997-1011, 1 August 2008

CIT, 2008c. Bone marrow micronucleus test by intraperitoneal route in mice. CIT 32753 MAS. 16 June 2008

ConsExpo 4.1. Consumer Exposure and Uptake Models. Available from the following internet site: www.rivm.nl/en/healthanddisease/productsafety/Main.jsp

ECHA, 2008 Guidance on information requirements and chemical safety assessment. ChapterR.11: PBT Assessment, May 2008. available online http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r11_en.pdf?vers=20_08_08

Hagendorfer et al., 2010. Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. J Nanopart Res 12, 2481–2494

Landsiedel, 2008. Short term inhalation tests with 8 nanomaterials in rats. Presentation at the annual SOT meeting (March 2008). Available from the following internet site: http://www.basf.com/group/corporate/en/sustainability/dialogue/in-dialogue-with-politics/nanotechnology/knowledge/safety-research

Landsiedel et al., 2010. Testing Metal-Oxide Nanomaterials for Human Safety. Adv. Mater., 22, 2601-2627

RCC, 2005a. Determination of the water solubility and the partition coefficient (n-octanol / water) of FAT 65080/B. RCC study no. 859223, 26 Sept. 2005

RCC, 2005b. [14C] FAT 65080 (ETH50). Percutaneous Penetration of Micronized [14C] FAT 65080 (ETH50) Through Rat and Human Split-thickness Skin Membranes (in-vitro). RCC study no. A00112, 03 August 2005

RCC, 2005c. Dermal Absorption of Micronized [14C] FAT 65080 (ETH50) in the Rat (in vivo). RCC study no. A22432

RCC, 2007a. FAT 65'080/F4-Hour Acute Inhalation Toxicity Study in Rats, RCC study no. B38698, 16 Nov 2007

RCC, 2007b. [14C] FAT 65080 (ETH50) Percutaneous Penetration of [14C] FAT 65080 (ETH50) Micronized According to Technical Specifications Through Rat and Human Split-thickness Skin Membranes (in-vitro). RCC study no. B23624, 01 Nov 2007

RCC, 2007c. [14C] FAT 65080 (ETH 50): Absorption, Distribution and Excretion of [14C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. B23613; 1 Nov 2007.

RCC, 2007d. [14C] FAT 65080 (ETH 50): Absorption, Distribution, Metabolism and Excretion of [^{14}C] FAT 65080 (ETH 50) After Oral Administration to Male Rats. RCC study no. A89280; 4 July 2007.

Sayes et al., 2007. Assessing toxicity of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary toxicity profiles. Toxicological Sciences, 97, 163-180

APPENDIX I

SAFETY ASSESSOR CERTIFICATION OF DOSSIER

Dossier: ETH50 as UV Filter for Sunscreen Products

This Dossier's Supplement has been prepared and reviewed by the undersigned and reflects accurately the testing results summarized herein to support the safe use of the product in cosmetics.

s47F [redacted]

i.A. s47F [redacted] (PhD)
Senior Regulatory Manager
Global Regulatory Affairs, Cosmetics, Aroma Chemicals and Biocides

BASF SE
Ludwigshafen, Germany

07 January 2010

MEC
ITEM No: 4.1
MEETING: 7 Dec 2006

OTC MEDICINES SECTION

Supplementary data for Diethylamino hydroxybenzoyl hexyl benzoate

Diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus) is a new UVA filter intended for use in listed sunscreen products in Australia. At the April 6th 2006 Meeting of MEC, the committee indicated that there were issues which needed to be addressed prior to approval of this new UVA filter. The issues were related to the absence of adequate data on: dermal and oral absorption, skin irritation potential on repeated administration, reproductive toxicity, genotoxicity and oestrogenic activity of the filter.

BASF Australia Pty Ltd have provided some additional information in response to requests by MEC.

Assessment

The sponsor was requested (MEC minutes, attachment 1) to provide the following additional information in support of their application:

- *In vivo* oral and dermal absorption studies (as the submitted *in vitro* dermal absorption study did not allow a reliable estimate of dermal absorption);
- An *in vivo* genotoxicity (mouse micronucleus) study;
- The two studies referred to in the sponsor's application that were not provided to the TGA (a second *in vitro* dermal absorption study using pig epidermal membrane and a repeated dose topical application study in guinea pigs);
- A repeated insult patch test in humans (to address concerns regarding the potential for skin irritation following repeated dermal exposure to the substance);
- Further reproductive studies, assessing general reproductive performance in rats and pre- and post-natal development in rats;
- A study assessing possible oestrogenic activity.

1. Dermal and oral absorption

Although the MEC committee requested the sponsor to provide an *in vivo* dermal absorption study because of concerns related to technical issues (including high variability not only in the individual values but also across studies) in the *in vitro* data, the sponsor has not submitted any *in vivo* dermal absorption study. However, the sponsor has submitted a further *in vitro* dermal absorption study in pig skin.

The *in vitro* dermal absorption study was conducted using standards of GLP and quality assurance. Although the study was conducted in the year 2003, it was not submitted to the OTCMS in the original submission. In this study, dermal absorption of an emulsion containing 10% diethylamino hydroxy-benzoyl hexyl benzoate was found to be only 0.042%, which was ~25 times lower than the quantity reported in the previously submitted *in vitro* study in pig skin (~1% of the administered dose was absorbed). It is noted that the two studies were conducted in different laboratories, which could perhaps explain the differences

in the results of the two studies. Moreover, there were a few technical issues in the previously assessed study (some membranes were found to be leaky and the data from the leaky membranes had to be excluded from analysis; high variability in the individual values; low recovery rate) and the results of the study were assessed to be questionable.

It is noted that both the *in vitro* dermal absorption studies (the newly submitted as well as the previously evaluated) were conducted with o/w emulsions containing 10% diethylamino hydroxybenzoyl hexyl benzoate. It appears that diethylamino hydroxybenzoyl hexyl benzoate, an oil soluble UVA filter, is incorporated in the oily phase of these emulsions and hence it is not clear whether absorption characteristics of diethylamino hydroxybenzoyl hexyl benzoate in the emulsion form would be similar to those of the substance if formulated differently as a non-emulsion (eg. formulated as a solution).

Based on the above, the submitted data on dermal absorption is not considered adequate.

The MEC also requested an *in vivo* oral absorption study to estimate the extent of oral bioavailability in some toxicity studies which were conducted with the oral route of administration (13-week repeat dose toxicity and developmental toxicity in rats). The data on oral absorption was also considered relevant since these studies were considered for estimating the margin of exposure (safety margin) of diethylamino hydroxybenzoyl hexyl benzoate (the no-effect level in the developmental toxicity was selected). The sponsor has not provided any study on oral absorption. The sponsor's agent (letter dated 25 August 2006) has stated that for reasons provided before (see previous evaluation report of OTCMS; the reason was mainly "lack of systemic effects after both acute and subchronic administrations"), the sponsor has not conducted any *in vivo* oral absorption study.

2. Skin irritation and sensitisation

Two single patch skin irritation studies in human subjects, although not using GCP or QA guidelines, were submitted (one conducted with the pure substance; the other with a formulation containing 10% diethylamino hydroxybenzoyl hexyl benzoate). In both these studies, a single topical administration of diethylamino hydroxybenzoyl hexyl benzoate did not cause any skin irritation.

It appears that both these studies have been submitted as alternatives to a repeated insult patch test. However, since the studies were single patch studies, the studies cannot be considered as equivalent to a repeated insult patch test (see also below).

The sponsor has now provided the full study report for the 14-day skin irritation study in guinea pigs (study conducted with GLP and QA). In this study, the vehicle (propylene glycol) and the test substance (10% and 20%) showed similar skin reactions suggesting that the test substance (10 or 20%) was not a skin irritant on repeated dermal application for 14 days.

A concern was raised in the earlier evaluation that the summary provided for this study suggested that the test substance had the potential to be a skin irritant on repeated application. Hence the MEC advised that the sponsor should provide a full study report for the 14-day guinea pig skin irritation study and also provide a repeated insult patch study in humans to address the issue. The submitted guinea pig study has now clarified that the skin reactions reported before in the study summary were due only to the vehicle and not the test substance.

However, the OTCMS evaluator's view is that the sponsor should have selected a suitable vehicle that does not show any skin irritation in the animal model used so that the effect of the vehicle does not interfere with the interpretation of the study results.

It is noted that in this study, open application (and not occlusive or semi-occlusive) was used for topical application of the test substance and hence it is not clear for how long the test substance was in contact with the skin. Moreover, it is noted that the animal model (guinea pigs) is not routinely used for testing skin irritation potential and rabbits (in the absence of a clinical study) are the preferred animal model for this purpose.

A repeated insult patch study in humans would be more relevant to address the potential of skin irritation (as well as sensitisation, see below) on repeated administration. Although the MEC requested the sponsor to submit a repeated insult patch study to address the issue of skin irritation on repeated administration, the sponsor has not provided such a study.

A repeated insult patch study would also address the potential of skin sensitisation. Although a sensitisation study in guinea pigs has been submitted before to address this issue, the guinea pig study has been recently criticised by the Scientific Committee on Consumer Products (SCCP, in EU; see attachment 2) in June 2006. The SCCP stated that "several questions may be raised concerning the study" and that "the study cannot be evaluated" although details of the issues have not been stated. The same study was previously evaluated by the previous EU committee, the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP; appears to be replaced in 2004 with the SCCP) but the committee did not raise any concerns regarding the quality of the study. Please note that the OTCMS evaluator pointed out before that in this study, both the vehicle (olive oil) and the test substance (25% concentration) showed skin reactions during the induction phase. However, since skin reactions were similar in the control and treated groups and there were no reactions during the challenge phase, the evaluator concluded that the test substance was not a skin irritant or a sensitiser in this study.

Using all available data on diethylamino hydroxybenzoyl hexyl benzoate, including that on *in vitro* dermal absorption, the SCCP (2006) calculated a margin of safety of 2150 (the margin of safety was previously calculated by the SCCNFP to be 6667 and by the OTCMS evaluator to be only 111; a safety margin of 100 is the minimum requirement) but concluded that:

> "Although this substance is presently permitted and used as a sunscreen, the SCCP is of the opinion that the information submitted is not conform [*sic*] to current standards and guidelines for the safety evaluation of cosmetic ingredients.

Before any further consideration, the following information is required:

- an absorbance spectrum of the substance
- a mammalian gene mutation test".

It is evident from the above that the SCCP does not consider available data on diethylamino hydroxybenzoyl hexyl benzoate as adequate. It is not clear from the conclusions of the SCCP whether the committee requires an *in vivo* mammalian genotoxicity assay. Please note that the mouse micronucleus test (clastogenicity assay rather than a mutation test), submitted to the OTCMS for evaluation (see below), has not been evaluated by the SCCP.

Because concerns were raised regarding the 14-day repeated application study in guinea pigs (vehicle and test substance showing skin reactions; open application), and regarding the skin sensitisation study in guinea pigs (concerns raised by SCCP), it is recommended that the sponsor submit a repeated insult patch study in humans. The study should be conducted using an appropriate vehicle (which preferably does not show any skin reactions) for the test substance. As stated before, the repeated insult patch study would address not only the issue of skin irritation on repeated administration, but also the issue of skin sensitisation.

3. Phototoxicity and photoallergy

The SCCP (2006) has commented that in a phototoxicity and photoallergenicity test in guinea pigs (previously submitted to OTCMS), two "very specific" wavelengths of UV irradiation were used (365 nm of UVA; 312 nm of UVB) without giving information of the absorption spectra of the substance. The SCCP commented that broadband UVA and UVB irradiation would be more appropriate to mimic the intended use of this cosmetic UV filter. The OTCMS evaluator's comment previously for this study was that the number of animals used was not adequate (only 10 in the treated group). In response to this comment, the sponsor's agent (letter dated 5 April 2006) replied that they have requested "that the client provide copies of (more comprehensive) phototoxicity/photosensitisation studies if they have been conducted". So far the study/studies have not been provided.

4. Genotoxicity

An *in vivo* micronucleus study in mouse was submitted, which met all current standards of GLP and quality assurance. Diethylamino hydroxybenzoyl hexyl benzoate at two intraperitoneal doses of ~2000 mg/kg/injection did not induce an increase in micronuclei above control levels indicating it was not genotoxic (clastogenic) in this assay. This *in vivo* result added to the negative results already seen in a series of *in vitro* assays assessing different end points confirming a low likelihood of genotoxic activity.

5. Endocrine function

In a Hershberger assay, treatment with a combination of testosterone (0.4 mg/kg/day) and diethylamino hydroxybenzoyl hexyl benzoate (1000 mg/kg/day but not 200 mg/kg/day) for 10 days showed a weak antiandrogenic effect on prostate (ventral) but other accessory sex organs were not affected by the combination. Liver weights were higher in the groups treated with testosterone (0.4 mg/kg/day + diethylamino hydroxybenzoyl hexyl benzoate 200 or 1000 mg/kg/day). There were no histological changes in the liver suggesting that the increased liver weights may be due to metabolic load caused by diethylamino

hydroxybenzoyl hexyl benzoate, and may not be due to direct toxicity. The no-effect level for this study was determined to be 200 mg/kg/day.

It is noted that in this assay, the individual effect of diethylamino hydroxybenzoyl hexyl benzoate (without combining with testosterone) on the organ weights was not tested, but this is not considered a major deficiency.

In a study to assess oestrogenic potential (uterotrophic) in young rats, diethylamino hydroxybenzoyl hexyl benzoate did not show any oestrogenic effect at 250 or 1000 mg/kg/day by gavage. Body weights were significantly lower at 1000 mg/kg/day. Based on this effect, the no-effect level was 250 mg/kg/day for diethylamino hydroxybenzoyl hexyl benzoate.

It is noted that in this study, only the oestrogenic potential was tested by administering the test substance alone. However, the anti-oestrogenic effect of diethylamino hydroxybenzoyl hexyl benzoate was not tested by co-administering diethylamino hydroxybenzoyl hexyl benzoate with an oestrogen. Hence, although the study can be designed to detect either oestrogenic or antioestrogenic potential of chemicals, the sponsor has addressed only one aspect of the potential interaction with oestrogenic receptors.

6. Reproductive toxicity

The MEC wanted additional studies on reproductive toxicity, since only a developmental toxicity study in rats has been submitted before. In the developmental toxicity study evaluated before, there were skeletal variations in the fetuses. On the basis of these effects, the OTCMS evaluator determined a NOEL (no observable effect level) of 40 mg/kg/day po. However, the sponsor's study report determined a NOAEL (no observable **adverse** effect level) of 200 mg/kg for maternal toxicity (transient salivation and reduction of food intake at 200 and 1000 mg/kg/day; decreased body weight gain at 1000 mg/kg/day) arguing that the skeletal variations were not treatment-related since the changes were not statistically significant (although the changes were above concurrent and historical control values).

The sponsor has not provided any study on general reproductive performance in rats, and on pre- and post-natal toxicity in rats. The sponsor has provided the Hershberger assay (above) to address this issue. This assay can address the potential of androgenic or antiandrogenic potential of a chemical, but cannot be considered as an alternative to the study on general reproductive performance or the study of pre- and post-natal toxicity in rats.

Summary/recommendation

Two *in vitro* studies on dermal absorption have been submitted so far. In one study, which had technical issues such as leaky membrane and low recovery (but leaky membranes were excluded from data analysis), dermal absorption was estimated to be ~1%. However, in the other study, absorption (0.042%) was ~25 times lower. An *in vivo* dermal absorption study, conducted with an appropriate vehicle for the test substance, is still required.

Both the *in vitro* studies were conducted with the test substance formulated as an emulsion. It is not clear whether absorption of the substance in an emulsion form would be different if the formulation was a non-emulsion (eg. solution). Although the sponsor has stated that

diethylamino hydroxybenzoyl hexyl benzoate can be readily incorporated in the oily phase of emulsions, it is not clear whether the chemical would be marketed in the form of an emulsion.

An oral absorption study was requested by the MEC, but the sponsor has not provided this study.

In two skin irritation studies in humans (single patch studies), diethylamino hydroxybenzoyl hexyl benzoate was not a skin irritant at a concentration of 10%.

Evaluation of a 14-day skin irritation study in guinea pigs confirmed the need for a repeated insult patch study in humans since there were issues related to the vehicle used (skin reactions), method of application (open application, not occlusive or semi-occlusive) and the animal model (rabbits preferred). A guinea pig skin sensitisation study suggested that the test substance (25%) was not a skin irritant or sensitiser, but also had concerns related to the use of vehicle which caused skin reactions during the induction phase. The SCCP in EU has commented that "several questions may be raised" concerning this sensitisation study and that the study "cannot be evaluated".

A repeated insult patch study, although requested by the MEC, has not been submitted. The study, if properly conducted, would address concerns regarding skin irritation on repeated administration as well as skin sensitisation potential.

The SCCP had concerns regarding the wavelengths of UV irradiation used in a phototoxicity and photoallergenicity study in guinea pigs. The OTCMS evaluator's comment for this study was that the number of animals used was not adequate. Hence, a new study may be required to address the potential of phototoxicity and photoallergenicity. Similarly, in relation to a previous concern that in photogenotoxicity studies metabolic activation was not used, the sponsor replied (5 April 2006) that they have requested the client to provide copies ("more comprehensive") of additional photogenotoxicity studies if any have been conducted. No additional photogenotoxicity studies have been submitted so far.

There are no studies on general reproductive performance, and pre-and post-natal toxicity in rats.

In an *in vivo* micronucleus study in mouse that has been submitted, diethylamino hydroxybenzoyl hexyl benzoate did not show clastogenicity at two daily intraperitoneal doses of ~2000 mg/kg/day. This *in vivo* result added to the negative results already seen in a series of *in vitro* assays assessing different end points suggesting low potential for genotoxicity.

In a Hershberger assay, a weak antiandrogenic effect was seen on prostate (ventral) but other accessory sex organs were not affected with a combination of testosterone (0.4 mg/kg/day) and diethylamino hydroxybenzoyl hexyl benzoate (1000 mg/kg/day; but not at 200 mg/kg/day).

In a study to assess the oestrogenic potential (uterotrophic) in young rats, diethylamino hydroxybenzoyl hexyl benzoate did not show any oestrogenic effect at 250 or 1000 mg/kg/day po but body weights were significantly lower at 1000 mg/kg/day. In this study, the anti-oestrogenic effect of diethylamino hydroxybenzoyl hexyl benzoate was not tested by co-administering diethylamino hydroxybenzoyl hexyl benzoate with an oestrogen.

The sponsor has only partially addressed the concerns of the MEC. Although the sponsor has submitted some of the requested studies, the following studies are still required to adequately address MEC's concerns and for further evaluation:

- an *in vivo* dermal absorption study in rats/humans;
- an *in vivo* oral absorption study in rats/humans;
- a repeated insult patch study in humans;
- a study on general reproductive performance in rats;
- a study on pre- and post- natal toxicity in rats;
- antioestrogenic potential in rats, or a suitable alternative assay; and
- phototoxicity and photoallergenicity
- photogenotoxicity studies conducted with metabolic activation

Based on the above deficiencies, the sponsor's application is **not supported**. The committee's opinion is requested.

EVALUATION OF SUBMITTED TOXICITY DATA

Dermal transport of 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoic acid hexylester from an emulsion across porcine skin *in vitro* (STP 017-00). Across Barriers GmbH, Germany; Report no. C-10106-081-0603; U. Bock; 19 September 2003; GLP/QA-yes.

The *in vitro* absorption of diethylamino hydroxybenzoyl hexyl benzoate from an o/w emulsion containing 10% diethylamino hydroxybenzoyl hexyl benzoate across pig skin was examined. The test formulation was applied to prepared skin membranes (dermatomised; ~500 µm thickness; n = 9 skin biopsies from 3 pigs) which were positioned across a chamber that contained a receptor fluid. The test material was applied at a nominal concentration of 2 mg/cm^2 (equivalent to 200 µg diethylamino hydroxybenzoyl hexyl benzoate/cm^2) and left in contact for a period of 24 hours at 32 ^{0}C. The rate and extent of absorption of the test substance were determined by removing samples of the receptor fluid at 0, 0.5, 1, 3, 6, 20 and 24 hours after application of the test substance. At the end of the experiment, the skin biopsies were separated into stratum corneum and deeper layers by tape stripping and distribution of diethylamino hydroxybenzoyl hexyl benzoate was monitored in different skin layers, including that remaining on the surface of the skin. The concentration of test substance in the receptor fluids, different layers of the skin, and in the donor and receptor washings was analysed by HPLC-PDA.

Caffeine (10 mg/mL; initial concentration in the donor compartment: 9.8 mg/mL; vehicle not stated) was used as a reference compound to study dermal absorption. For caffeine, only one experiment was conducted for each pig skin (dermatomised), which had a thickness of 1000 µm.

The results revealed that the 24-hour cumulative absorption of the emulsion containing 10% diethylamino hydroxybenzoyl hexyl benzoate across the skin was 0.042%. For caffeine, the extent of absorption (%) was not stated but the mean 24-hour cumulative transport across the skin was reported to be 51.3 µg/cm^2. The mean permeability coefficient (P_{app}) for caffeine was 7.2 E-08 cm/s and the cumulative recovery (presumably absorbed + non-absorbed) was 102.2%. The concentration of caffeine in different skin layers was not determined.

In vitro dermal absorption of diethylamino hydroxybenzoyl hexyl benzoate in 24 hours

	Amount of diethylamino hydroxybenzoyl hexyl benzoate	
	µg/cm^2 of skin surface mean ± SD	***% of applied dose mean ± SD***
Donor compartment	202.1 ± 40.2	*91.9 ± 4.8*
Stratum corneum	1.7 ± 1.3	*0.8 ± 0.6*
Deeper skin	0.10 ± 0.12	*0.042 ± 0.050*
Receptor compartment	0	*0*
Total recovery	*203.9 ± 4.04*	*92.7 ± 4.8*

n = 9 (9 skin specimens from 3 pigs). Diffusion area of each skin sample: 3 cm^2.

It is noted that the formulation tested was an oil in water emulsion containing 10% diethylamino hydroxybenzoyl hexyl benzoate in the oil phase. It is not clear whether the absorption of the substance in an emulsion form would be different if the formulation was a non-emulsion (eg. solution).

Under the conditions of this *in vitro* study, 10% diethylamino hydroxybenzoyl hexyl benzoate had low dermal absorption (<0.1% of the applied dose in 24 hours).

Uvinul A Plus - Study of skin irritation in guinea pigs. Open application to the intact skin (14 applications). BASF Aktiengesellschaft, Germany; Project no. 99H0636/02074; A.O. Gamer; 26 March 2004; GLP/QA-yes.

In guinea pigs, diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus; vehicle: propylene glycol) was tested for its potential to induce skin irritation after 14 open applications (50 µL to the intact skin to the right flank, 4 cm^2 area; the application area was clipped free of hair) over a study period of 2 weeks. Two groups of guinea pigs (3/sex/group) were used and the test substance (10% or 20% in propylene glycol to the right flank) or the vehicle (to the left flank) was applied daily for two weeks. Each animal was treated with only one concentration of the test substance and the vehicle. Skin reactions were monitored (Draize scale) at 24 hours after each application.

Application of the vehicle or the test substance (10% or 20%) caused skin reactions in all the animals: very slight erythema (barely perceptible; score = 1) or well-defined erythema (score = 1). There were no dose-dependent effects for the test substance. Since the skin reactions were comparable for the vehicle and the test substance (suggesting that the reactions were due to the vehicle), and the test substance did not show concentration-dependent changes, the test substance (10% or 20% diethylamino hydroxybenzoyl hexyl benzoate) was not considered a skin irritant in the study.

Cytogenetic study *in vivo* with Uvinul A Plus in the mouse micronucleus test after two intraperitoneal administrations. BASF Aktiengesellschaft, Germany; Project no. 26M0636/024140; G. Engelhardt and E. Leibold; 10 April 2003; GLP/QA-yes.

The purpose of this study was to determine whether diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus) had the potential to induce the formation of micronuclei in polychromatic erythrocytes (PCE) extracted from the bone marrow of NMRI mice. Groups of mice (5/sex) were exposed to Uvinul A Plus and control agents prior to assessment of the occurrence of micronuclei in PCE's from bone marrow. The presence of micronuclei was evaluated in 2000 PCE's/animal. The test material in DMSO was administered as two intraperitoneal injections (24 hours apart; volume 4 mL/kg) at 500, 1000 or 2000 mg/kg (per injection), with bone marrow collection conducted at 24 hours after the second dose. Doses used in this study had been established in a pre-study range-finding assay. In the pre-test, piloerection, squatting posture and "poor" health were clinical signs with no distinct symptomatic differences between the male and female animals. Hence the main study was conducted only in male animals (see notes below). Assessment of possible cytotoxicity was determined using the PCE to normochromatic erythrocyte (NCE) ratio as an index. Also, the PCE numbers in treated groups and vehicle controls were compared. DMSO was used as the vehicle (given alone in control group) to deliver the test material and two positive control groups were given either a single injection of 20 mg/kg cyclophosphamide IP or 0.15 mg/kg vincristine IP.
Animals survived dosing with limited clinical signs of toxicity; these consisted of piloerection and squatting posture, for all the three doses of diethylamino hydroxybenzoyl hexyl benzoate, and "poor general state" for the highest dose. Cytotoxicity was evident, with PCE/NCE ratios decreasing especially for the 2000 mg/kg group. There was no evidence of clastogenicity for diethylamino hydroxybenzoyl hexyl benzoate: statistical analysis of the data for the incidence of PCE micronuclei revealed that none of the test item groups showed

any significant variation from the vehicle, while the positive controls were significantly ($p<0.01$) different to the vehicle control.

Results for the micronucleus assay are summarised in the following table:

Test group	**Dose mg/kg**	**Total number of PCEs** (for 5 animals)	**Total number of NCEs** (for 5 animals)	**PCE's with micronuclei**	**Ratio of PCEs to NCEs**
Vehicle	0	10,000	4044	1.4%	2.5
Test item	500	10,000	4830	1.4%	2.1
Test item	1000	10,000	5116	1.6%	2.0
Test item	2000	10,000	6775	1.1%	1.5
Positive control-CPP	20	10,000	3961	18.5%**	2.5
Positive control-VCR	0.15	10,000	4351	76.6%**	2.3

CPP = cyclophosphamide; VCR = vincristine.
** $p<0.01$, compared to the control group (Wilcoxon Test, one-sided).

The study also investigated the stability of the test substance over a period of 4 hours. Three samples of each dose were taken from the test substance preparations, kept at room temperature until the treatment of the last animal (~1 hour) and then deep-frozen until they were evaluated analytically (HPLC). The results indicated that 82-85% of the theoretical values were found in the samples tested implying that the administered doses for the test substance were in fact 15-18% lower than the intended values.

Overall, a comparison between test groups and the vehicle control showed there was no statistical significant or biologically relevant difference in the rate of formation of micronuclei. The incidences of micronuclei induction occurred at a similar frequency in test groups and vehicle controls. Results from the positive control groups showed significantly greater induction of micronuclei compared with both the test groups and the vehicle control. Diethylamino hydroxybenzoyl hexyl benzoate was not clastogenic in this assay.

Notes: According to the OECD guidelines (no. 475), the test should be conducted with at least 5 analysable animals per sex. However, the guidelines also state that if there are data to show that there are no substantial differences in toxicity between sexes, then testing in a single sex will be sufficient.

> Human patch test on cosmetic ingredients. Japan Hair Sciences Association. Project no. 15070; Dr Nagashima; 12 March 2004; GCP/QA-no.

In a single patch study conducted in human subjects (18 males and 26 females), 0.01 g of diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus) was applied occlusively for 24 hours using a Finn chamber. Skin reactions were monitored at 1 and 24 hours after patch removal. Except for erythema (score: +) in one subject (out of 44 subjects) at 1 hour after patch removal, there were no reactions at 1 or 24 hours after patch removal. Diethylamino hydroxybenzoyl hexyl benzoate is assessed to be a non-irritant in this study.

> Human patch test on cosmetic ingredients. Japan Hair Sciences Association. Project no. 15058; Dr Nagashima; 5 February 2004; GCP/QA-no.

In a single patch study conducted in human subjects (17 males and 28 females), 0.01 g of "Uvinul A Plus milk 056-13" containing 10% diethylamino hydroxybenzoyl hexyl benzoate was applied occlusively for 24 hours using a Finn chamber. Skin reactions were monitored at 1 and 24 hours after patch removal. There were no reactions in any subject at 1 or 24 hours after patch removal. "A Plus milk 056-13" containing 10% diethylamino hydroxybenzoyl hexyl benzoate was not an irritant in this study.

> Uvinul A Plus. Hershbergher assay in Wistar rats. BASF Aktiengesellschaft, Germany; Project no. 47S0495/00149; U. Kaspers *et al.*; 15 October 2003; GLP/QA-yes.

In a study to assess the androgenic or anti-androgenic potential, Uvinul A Plus was administered to groups of castrated young (56 or 57 days old) male Wistar rats (6 animals/group) at 0 (vehicle: corn oil), 200 or 1000 mg/kg/day by gavage for 10 days. The animals also received testosterone propionate (0.4 mg/kg/day; vehicle: corn oil) subcutaneously during this period. Another group (vehicle control) was treated with corn oil orally as well as subcutaneously. Clinical signs and body weights were monitored daily. Food consumption was measured on treatment days 5 and 9. At the end of the treatment, the animals were sacrificed after a fasting period of 16-20 hours, and blood was collected to determine levels of LH, testosterone and dihydrotestosterone by radio-immunoassay. Gross pathology was conducted. The weights of ventral prostate, seminal vesicle (with coagulation gland), levator ani muscle with bulbocavernosus muscle (Musculus levator ani with Musculus bulbocavernosus), bulbo-urethral gland, glans penis, liver, kidneys and adrenal glands were recorded. Histological examination was conducted on ventral prostate, seminal vesicles (with coagulation gland), bulbo-urethral gland and gross lesions in genital organs.

Diethylamino hydroxybenzoyl hexyl benzoate did not show any treatment-related effect on clinical signs, food consumption or body weight.

As expected, blood levels of testosterone were significantly increased after treatment with testosterone alone (by ~3.5 fold when compared to vehicle control). Combining testosterone with diethylamino hydroxybenzoyl hexyl benzoate (1000 mg/kg/day) did not significantly alter the effect of testosterone alone (testosterone levels: 2.78 and 3.16 nmol/L in the absence and presence of diethylamino hydroxybenzoyl hexyl benzoate). Administration of testosterone alone lowered (statistically non-significant) the levels of LH and dihydrotestosterone (by 21% and 36%, respectively). Diethylamino hydroxybenzoyl hexyl benzoate did not modify the effect of testosterone on these hormone levels.

After treatment with testosterone alone, the androgenic effect was evident as significantly increased absolute and relative weights of all the five sex accessory organs/tissues (see table below). Although combination with diethylamino hydroxy-benzoyl hexyl benzoate did significantly alter the effect of testosterone on these organ/tissue weights (when tested by Kruskal-Wallis-H-+Wilcoxon test), the absolute and relative weights of ventral prostate were found to be lower (relative weights by 19% although statistically non-significant when compared to testosterone alone) in the group treated with 1000 mg/kg/day of diethylamino hydroxybenzoyl hexyl benzoate suggesting a weak antiandrogenic effect.

The individual effect of diethylamino hydroxybenzoyl hexyl benzoate (without combining with testosterone) on the organ weights was not tested.

The relative weights of liver were significantly increased when diethylamino hydroxybenzoyl hexyl benzoate (by 10% and 17% at 200 and 1000 mg/kg/day, respectively) was co-administered with testosterone.

Except for immaturity of seminal vesicle, bulbo-urethral gland and ventral prostate in the group treated with vehicle control group (immaturity of these organs are expected since the animals were young and castrated; the other groups, although young and castrated, were treated with testosterone with or without the test substance), there were no gross pathological or histological changes. There were no gross pathological changes in the liver except for focal constriction in one animal that occurred only in the vehicle control group.

Changes in organ/tissue weights

	Vehicle	**Testosterone**	**Testosterone + DHHB 200 mg/kg/day**	***Testosterone + DHHB 1000 mg/kg/day***
Body weights	208.6	218.2	218.4	*208.8*
Absolute weights				
Ventral prostate (mg)	17.3**	97.2	96.8	*79.2*
Seminal vesicle (mg)	25.7**	193.4	179.8	*185.8*
Bulbo-urethral gland (mg)	5.6**	21.1	25.3	*22.0*
Glans penis (mg)	40.8**	65.9	61.5	*62.2*
Levator ani muscle (mg)	149.7**	324.3	305.8	*316.4*
Adrenal glands (mg)	62.5	62.7	55.3	*62.0*
Liver (g)	6.07	6.28	6.90	*7.06*
Kidneys (g)	1.579	1.646	1.735	*1.610*
Relative weights (%)				
Ventral prostate	0.008**	0.045	0.044	*0.038*
Seminal vesicle	0.012**	0.089	0.082	*0.090*
Bulbo-urethral gland	0.003**	0.010	0.011	*0.011*
Glans penis	0.02**	0.030	0.028	*0.030*
Levator ani muscle with bulbocavernosus muscle	0.071**	0.149	0.140	*0.152*
Adrenal glands	0.030	0.029	0.025	*0.030*
Liver	2.91	2.88	3.16**	*3.38***
Kidneys	*0.759*	*0.755*	*0.794*	*0.771*

DHHB = diethylamino hydroxybenzoyl hexyl benzoate. **p<0.01, compared to the group which received testosterone alone (Kruskal-Wallis-H-+Wilcoxon test, two sided for comparing DHHB with testosterone alone, or Wilcoxon test, two sided for comparing vehicle control with the testosterone group). n = 6/group.

Stability testing of diethylamino hydroxybenzoyl hexyl benzoate in corn oil indicated that the substance was stable (recovery: 102-105% of the nominal concentration) during the administration period.

In summary, diethylamino hydroxybenzoyl hexyl benzoate showed a weak antiandrogenic effect on prostate (ventral) at a dose of 1000 mg/kg/day but other accessory sex organs were not affected. Under the conditions of the study, the no-effect level was 200 mg/kg/day.

> 2(-4-Diethylamino-2-hydroxybenzoyl)-benzoesaurehexylester (DHE). Uterotrophic assay in immature female Wistar rats. Oral administration (gavage). BASF Aktiengesellschaft, Germany; Project no. 07R0228/99121; Dr. Kaspers. 13 July 2001; GLP/QA - nil.

In a study to assess the oestrogenic potential, Uvinul A Plus was administered to groups of young (20 or 21 days old) female Wistar rats (10 animals/group) at 0 (vehicle: olive oil), 250

or 1000 mg/kg/day by gavage for 3 days. A positive control group (n = 10) received a single administration (gavage) of 5 µg/kg of diethylstilbestrol dipropionate. Body weights and clinical signs were monitored everyday. Approximately 20-24 hours after the last administration, the animals were anaesthetised with CO_2 and sacrificed. The uteri were removed, weighed and histological examination was conducted.

There were no clinical signs. Body weight gain was significantly lower on day 0-1 (24 hours after the first gavage administration) in the group treated with 1000 mg/kg/day (weight gains were 4.0, 3.1 and 1.8 g in the control, 250 and 1000 mg/kg/day groups, respectively). The overall body weight gain at the end of the treatment period was significantly lower in the HD group (11.0, 10.2 and 8.4 g in the control, LD and HD groups, respectively). Uterine weights and uterine histology were not affected in the groups treated with diethylamino hydroxy-benzoyl hexyl benzoate. The positive control group showed appropriate changes in uterine weight (higher by ~5 fold) and histology (enlargement of endometrial wall; elongation of epithelia, luminal surface; expanded lumina) which validated the study method.

Based on the effect on body weight, the no-effect level was 250 mg/kg/day for diethylamino hydroxybenzoyl hexyl benzoate.

OTCMS
October 2006

Attachment 1

Extract of MEC Minutes 6 April 2006

4.1 Diethylamino hydroxybenzoyl hexyl benzoate

This was an application by BASF Australia Pty Ltd for approval of diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus) for use as a UVA filter in listed sunscreen products and cosmetics at concentrations of up to 10%.

Diethylamino hydroxybenzoyl hexyl benzoate was approved by the European Union in January 2005 for use in sunscreen products at concentrations of up to 10%. That approval was based on an assessment by the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) of the European Commission.

The sponsor advised that diethylamino hydroxybenzoyl hexyl benzoate is "yet to be approved" for use in New Zealand, Canada, USA, UK or Sweden, and that, as it has not yet been marketed, no adverse events have been reported. The sponsor asserted that no adverse effects are known and none would be suspected, based on the studies presented.

Pharmaco/toxico-kinetics

An *in vitro* study reported an overall percutaneous absorption through pig epidermal membranes of ~1% of the test substance over 24 hours. The evaluator queried the reliability of the study, due to technical problems such as leaky membranes and high variability in the individual data, and had noted that the SCCNFP did not consider the study to be valid for similar reasons. A summary of a second *in vitro* dermal absorption study (that was submitted to the SCCNFP, but not to the TGA) reported a lower percutaneous absorption of 0.04%. The results of the two *in vitro* studies showed high variability, both within one study, and between the studies. The evaluator considered that the *in vitro* studies may not be reliable, and had recommended that an *in vivo* dermal absorption study should be provided. The evaluator had also recommended the provision of an oral absorption study, to allow assessment of exposure associated with the oral repeat dose and reproductive toxicity studies in rats.

No data were provided on the metabolism of diethylamino hydroxybenzoyl hexyl benzoate. The sponsor had indicated that specific ADME (absorption, distribution, metabolism and elimination) studies with radioactive material have not been performed, as the substance would have low toxicity or low absorption and metabolites were unlikely to be mutagenic or carcinogenic. The evaluator has not accepted the sponsor's arguments, but contended that there would be at least limited bioavailability after oral exposure, and noted that a developmental toxicity study suggested there is some oral absorption. Systemic toxicity may also occur following dermal exposure.

The SCCNFP had calculated a safety margin of 6667 (based on a maximum absorption through the skin of 0.1 µg/cm^2 and a NOAEL of 200 mg/kg in a rat developmental study). The TGA evaluator had calculated a lower safety margin of 111, assuming dermal absorption of ~1%, based on the *in vitro* dermal study (assessed to be unreliable) submitted to the TGA.

Interaction with other UV filters

No data were provided on possible interactions between diethylamino hydroxybenzoyl hexyl benzoate and other UV filters, and information on its stability was in isolation from other UV filters. The substance was resistant to degradation under extremes of pH and temperature for up to 3 months, and was not photodegraded significantly after up to 6 hours of UV irradiation.

Local tolerance

The TGA evaluator classified diethylamino hydroxybenzoyl hexyl benzoate as a slight eye irritant, based on data using rabbits, but had noted that eye irritation may not occur at the intended concentration of 10% in topical products.

Diethylamino hydroxybenzoyl hexyl benzoate was not a primary skin irritant after a single application in rabbits, and did not appear to be a skin irritant after a single application (at up to 25%) in guinea pigs. A concentration of 25% did not induce skin hypersensitivity in guinea pigs. While the substance did not show a sensitisation or photosensitisation response in studies in guinea pigs, the evaluator had questioned the validity of the photosensitisation study. The evaluator had concluded (based on information reported in the sponsor's Product Safety document about a repeat-dose dermal irritation study in guinea pigs that was not provided to the TGA or evaluated by the SCCNFP) that repeated application may cause slight skin irritation at the intended concentration of 10%.

No local tolerance studies in human subjects were provided. The evaluator had recommended that a repeated insult patch test in humans should be provided, to address concerns regarding the potential for skin irritation following repeated dermal exposure (as seen in guinea pigs).

Acute/repeat dose toxicity

An acute oral toxicity study in rats showed that diethylamino hydroxybenzoyl hexyl benzoate has low acute toxicity (>2 g/kg). The NOEL (no-effect level) in a 13-week repeat dose oral toxicity study in rats was determined to be the high dose (1350 mg/kg/day). The evaluator noted that the observed increases in liver weight and changes in plasma bilirubin, together with toxic effects seen in a developmental toxicity study in rats, suggested some degree of systemic exposure following oral dosing. The extent of oral bioavailability in the rat could not be estimated, as kinetic data were not provided.

Reproductive toxicity

The evaluator had determined a NOEL of 40 mg/kg/day (the low dose) in a developmental toxicity study in rats, based on maternal and foetal toxicity (skeletal variations) seen at the medium and high doses (200 and 1000 mg/kg/day). The sponsor did not consider these to be toxic effects, and had determined NOELs of 200 mg/kg/day for maternal toxicity and 1000 mg/kg/day for developmental toxicity. There was no assessment of possible oestrogenic activity.

Genotoxicity

Four *in vitro* genotoxicity assays produced negative results, indicating that diethylamino hydroxybenzoyl hexyl benzoate was not genotoxic *in vitro* in the presence or absence of UV

radiation. The evaluator had recommended that an *in vivo* genotoxicity study (mouse micronucleus assay) should also be submitted, to address concerns about the potential genotoxicity of metabolites of diethylamino hydroxybenzoyl hexyl benzoate.

Cytotoxicity

Diethylamino hydroxybenzoyl hexyl benzoate was cytotoxic *in vitro* at 50 and 100 µg/mL. Comparison of results for the test substance and untreated control showed no evidence of phototoxicity.

Carcinogenicity

No carcinogenicity studies were submitted but the sponsor provided an argument addressing potential carcinogenicity. The committee noted the discussion in the evaluation report of the sponsor's argument. The evaluator contended that, overall, the sponsor had not provided adequate justification for not providing a long-term carcinogenicity study, and had noted the following issues:

- The submitted *in vitro* dermal absorption study in pig skin was inconclusive, a second *in vitro* study (also using pig skin) was not provided to the TGA, and the evaluator had recommended that an *in vivo* dermal absorption study should be provided (refer to *Pharmaco/toxico-kinetics*, above);
- As there were no direct data on metabolism (ADME study) of diethylamino hydroxybenzoyl hexyl benzoate, the potential for the formation of active metabolites is not accurately known;
- A repeated dermal irritation study in guinea pigs was not submitted, the limited dermal irritation data indicated that a 10% concentration of the substance may produce skin irritation on repeated administration, and the evaluator had questioned the validity of the study results of a photoallergenicity study in guinea pigs;
- No clinical skin irritation or skin sensitisation studies (human) were provided;
- There could be a non-genotoxic mechanism for carcinogenesis involving chronic skin irritation and inflammation – the animal skin irritation studies indicated that, while diethylamino hydroxybenzoyl hexyl benzoate may not be an irritant at a concentration of 10% after a single administration, it could be an irritant on repeated dermal exposure;
- Toxicity studies were of short duration (up to 13 weeks) and may not detect carcinogenicity potential;
- Genotoxicity was not adequately addressed, as *in vivo* genotoxicity data were not provided;
- As the *in vitro* photomutagenicity or photoclastogenicity assays did not include metabolic activation, it is not known whether photogenotoxic metabolites are formed;
- Diethylamino hydroxybenzoyl hexyl benzoate was shown to be cytotoxic in a cytotoxicity assay and *in vitro* genotoxic assays; and
- Human experience with the substance is limited, given that it was only approved in Europe in January 2005.

Discussion

The evaluator had recommended that additional data should be provided before a decision can be made regarding this application:

- *In vivo* oral and dermal absorption studies (as the submitted *in vitro* dermal absorption study did not allow a reliable estimate of dermal absorption);
- An *in vivo* genotoxicity (mouse micronucleus) study;
- The two studies referred to in the sponsor's application that were not provided to the TGA (a second *in vitro* dermal absorption study using pig epidermal membrane and a repeated dose topical application study in guinea pigs);
- A repeated insult patch test in humans (to address concerns regarding the potential for skin irritation following repeated dermal exposure to the substance);
- Further reproductive studies, assessing general reproductive performance in rats and pre- and post-natal development in rats;
- A study assessing possible oestrogenic activity.

The committee noted the sponsor's argument (in the response to the evaluation report) regarding the NOEL and safety margin calculated by the TGA evaluator for diethylamino hydroxybenzoyl hexyl benzoate. The sponsor's agent advised that the sponsor has been asked to provide further data, if available, including the second *in vitro* dermal absorption study, data addressing oestrogenic activity and a mouse micronucleus study.

A member raised concerns that use of sunscreens in Australia differs substantially from their use in Europe. As there is a much higher exposure to sunscreens in Australia than in Mediterranean countries, for example, as consumers are encouraged to apply sunscreens every day, the potential for adverse effects following dermal absorption of sunscreens is higher in Australia.

The committee recommended rejection of the application by BASF Australia Pty Ltd for approval of diethylamino hydroxybenzoyl hexyl benzoate for use as a UVA filter in sunscreen products and cosmetics, pending consideration of further data from the sponsor addressing the concerns that were raised in the evaluation report. An evaluation of these additional data should be returned to the MEC for further consideration.

Scientific Committee on Consumer Products, SCCP (2006). Opinion on benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester. COLIPA no S83. Adopted by the SCCP during the 8th plenary meeting of 20 June 2006. SCCP/0996/06.

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers. It appears that the SCCNFP was a former committee of the EC and was replaced in 2004 with the Scientific Committee on Consumer Products (SCCP).

Maximum (not the mean) absorption through the skin: 0.31 µg/cm^2; typical body weight of 60 kg for humans; a skin surface area of 18000 cm^2;
systemic exposure dose/kg bw = 18000 (cm^2) x 0.31 (µg/cm^2) ÷ 60 (kg) = 93 µg/kg or 0.093 mg/kg.
Using a NOAEL of 200 mg/kg, the margin of safety = 200/0.093 = 2150.

Salivation at 200 mg/kg/day was not considered as a toxic effect by the sponsor's study report.

Composition of the formulation (%): Diethylamino hydroxybenzoyl hexyl benzoate (10.0); ceteareth-6 and stearyl alcohol (2.0); ceteareth (2.0); dimethicone (3.0); glyceryl stearate SE (12.0); caprylic/capric triglyceride (7.0); PPG-3 myristyl ether (5.0); glycerine (3.0); disodium EDTA (0.1); DMDM hydantoin (0.3) and "water den." (55.6).

The sponsor's earlier submission stated that the 200 µg/cm^2 concentration was a dose level reflecting application conditions according to the recommendations of the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP).

Score: +? for light erythema; + for erythema; ++ for erythema, infiltration and papule; +++ for erythema, infiltration, papule and vesicula and +++ for bulla.

Composition: 10% diethylamino hydroxybenzoyl hexyl benzoate; 2% isopropyl isostearate; 2% cetyl alcohol; 1% glyceryl stearate SE; 1% ceteareth-25; 1% ceterareth-6 and stearyl alcohol; 0.1% methylparaben; 0.1% butylparaben; 0.1% disodium EDTA and 82.7% water.

Score: +? for light erythma; + for erythema; ++ for erythema, infiltration and papule; +++ for erythema, infiltration, papule and vesicula and +++ for bulla.

The animals (Wistar rats CrlGlxBrlHan:WI) were castrated 11 days prior to treatment.

CRL:WI (GLX/BRL/HAN) IGS BR rats were used.

MEC
ITEM no:
MEETING: 26/2/2009

OTC MEDICINES SECTION

Supplementary data (second) for Diethylamino hydroxybenzoyl hexyl benzoate

1 INTRODUCTION

Diethylamino hydroxybenzoyl hexyl benzoate (referred to as "Uvinul A Plus", the trade name, in this report) is a new UVA filter intended for use in listed sunscreen products at a concentration of up to 10% in Australia. The MEC have considered the sponsor's application before at two meetings, on 6 April 2006 and 7 December 2006, and commented that there were still issues which needed to be addressed prior to approval of this new UVA filter (see Attachments 1 and 2 for the minutes of the meetings held on 6 April 2006 and 7 December 2006, respectively). The issues were related to the absence of adequate data on: dermal and oral absorption, skin irritation potential on repeated administration, photosensitisation, reproductive toxicity and genotoxicity of the filter.

Specifically, the Committee wanted the following studies to be submitted:

- A 2-generation reproductive toxicity study in rats;
- A mammalian cell mutation assay (*in vitro*);
- A repeated insult patch test in humans;
- An *in vivo* dermal absorption study in rats, or alternatively an *in vitro* dermal absorption study conducted with human skin;
- An *in vivo* oral absorption study in rats; and
- A photosensitisation study in guinea pigs conducted with adequate number of guinea pigs.

BASF Australia Pty Ltd have now provided the required studies.

2 INTERNATIONAL STATUS

According to the sponsor, Uvinul A Plus is approved at a concentration of up to 10% for use in sunscreen products in the EU (28 January 2005), Switzerland (15 Nov 2006), China (1 January 2007), Japan (18 October 2005), Taiwan (July 2005), Korea (December 2007), ASEAN (September 2007) and New Zealand (in Cosmetic Products Group Standard 2006). Uvinul A Plus (10%) has also been approved in South America, Mexico, India, and South Africa (approval dates not stated). In addition, Uvinul A Plus has been reported to be approved in "many other countries which generally follow the cosmetic regulation of the EU".

In the EU, Uvinul A Plus had been considered by the Scientific Committee on Consumer Products (previously SCCNFP) in the year 2003. Based on the review of scientific data, approval was given in January 2005 for its use in sunscreen products. Subsequently, the SCCP re-evaluated the substance to determine whether Uvinul A Plus could also be safe in

cosmetic products other than sunscreen products at a concentration of up to 10%. After evaluating the substance, the SCCP recently (15 April 2008) concluded that the substance is safe for inclusion in other cosmetic products. The SCCP commented that "Based on the information provided, the SCCP is of the opinion that the use of diethylamino hydroxybenzoyl hexyl benzoate at a maximum concentration of 10% w/w in cosmetic products, including sunscreen products, does not pose a risk to the health of the consumer. Only the dermal application of diethylamino hydroxybenzoyl hexyl benzoate was considered. Due to lack of data with regard to inhalation exposure and toxicity, the safety of applications which would result in consumer exposure via the inhalation route could not be assessed".

3 ASSESSMENT

3.1 Pharmacokinetics

3.1.1 Dermal and oral absorption

The sponsor has submitted an *in vivo* dermal absorption study conducted in rats and two *in vitro* studies using rat and human skin. In addition, the sponsor has provided an *in vivo* oral absorption study in rats.

Two *in vitro* studies in pig skin membrane were submitted before (in earlier submissions) but the studies were found to have issues such as leaky membrane, high variability of individual values and/or low recovery. In one of these studies, the total recovery of the applied substance (Uvinul A Plus) from all sample collections (reservoir fluid, amount present in the skin, washings etc.) was 92% and the amount of dermal absorption was estimated to be 0.042% but in the other study (which had leaky membranes) the estimate of dermal absorption was high (1%).

The two *in vitro* studies recently submitted are useful in comparing dermal absorption in rat and human skin membranes. Based on the results, the sponsor has estimated skin absorption (amount recovered in 18 tape strips of stratum corneum, deeper layers of skin and receptor compartment; reported to be the "proportion of absorbed substance") to be 10.3% and 0.54% in rat and human skin preparations, respectively, thus showing dermal absorption of Uvinul A Plus from human skin is ~ 20 times lower than that of rat skin. Although the sponsor has included the fraction of Uvinul A Plus found in stratum corneum in the estimate of dermal absorption, it is not clear whether the amount in stratum corneum (at the end of 24 hour exposure) is available for systemic absorption. If the fraction in stratum corneum is excluded, the total amount recovered in deeper layers of skin and the receptor compartment was only 2.69% and 0.13% in rat and human skin, respectively. However, irrespective of whether stratum corneum is included or not in the estimate of dermal absorption (systemic exposure), the results showed that absorption of Uvinul A Plus from human skin was ~ 20 times below that of rats. The sponsor has therefore requested the OTCMS to consider the lower absorption of Uvinul A Plus in humans while estimating the safety margin of Uvinul A Plus.

An *in vivo* dermal absorption study in male rats showed that the absorption of Uvinul A Plus (10%) was ~ 3%. The extent of *in vivo* absorption of Uvinul A Plus is comparable to *in vitro* data in rat skin if the amount present in stratum corneum is excluded from the calculation (absorption: 2.69%). Since the *in vitro* studies showed that the dermal absorption of Uvinul

A Plus (for systemic exposure) in human skin was 20 times lower than that of rat skin, the *in vivo* dermal absorption in humans is likely to be ~ 0.15%.

In both the *in vitro* studies (human and rat skin) of the current submission, there were a few membranes which showed total recovery outside the limit of 100 ± 15%, but these values which deviated from the acceptable limits were excluded from data analysis.

In the rat oral absorption study (*in vivo*), oral absorption of radiolabelled Uvinul A Plus was ~ 40% in 72 hours after a single administration of 100 mg/kg with absorption ~ 2 times higher in females when compared to that in males (~ 24% in males and 54% in females).

3.2 Local tolerance

In the previous two evaluations, Uvinul A Plus was not found to be a skin irritant in humans after a single topical application of the intended concentration of 10%. The undiluted substance was not a skin irritant in rabbits. However, other studies (in guinea pigs) did not adequately address the potential of skin irritation and/or sensitisation on repeated administration and the MEC recommended submission of a repeated insult patch study (RIPT) in humans to address this issue.

The sponsor has now submitted an RIPT in humans. The study showed that a formulation containing 10% Uvinul A Plus was not a skin irritant or sensitiser.

To address another previous concern that an adequate number of animals was not used in a guinea pig study on phototoxicity and photoallergy, the sponsor has provided a new study conducted with adequate number of animals (n = 20 in the treated group). The new study (as well as the previous study with inadequate number of animals) showed that Uvinul A Plus is not a photoirritant or a photosensitiser at a concentration of 10%.

The undiluted substance was previously assessed to be an eye irritant in rabbits but at the intended concentration of 10%, eye irritation potential is likely to be minimal.

3.3 Acute and repeat dose toxicity

In studies submitted previously, Uvinul A Plus was found to be of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg) and , the no-observed-effect level (NOEL) for repeat dose toxicity in rats (in a 13-week repeat dose oral toxicity study) was 1350 mg/kg/day (mean of 1249 and 1452 mg/kg/day in males and females, respectively).

3.4 Reproductive toxicity

The MEC previously wanted the sponsor to submit additional studies on reproductive toxicity, since only a developmental toxicity study in rats has been submitted before. In the developmental toxicity study evaluated before, there were skeletal variations in the fetuses at 200 and 1000 mg/kg/day and these changes were seen in the presence of maternotoxicity. On the basis of these changes, the OTCMS evaluator determined a NOEL of 40 mg/kg/day po, the LD. However, the sponsor's study investigators determined a NOAEL (no observed **adverse** effect level) of 200 mg/kg for maternal toxicity (transient salivation and reduction of food intake at 200 and 1000 mg/kg/day; decreased body weight gain at 1000 mg/kg/day) and 1000 mg/kg/day for developmental toxicity arguing that the skeletal variations were not

treatment-related since the changes were not statistically significant (although the changes were above concurrent and historical control values).

The sponsor has now provided a 2-generation reproductive toxicity study in rats which also covers fertility and general reproductive performance as well as pre- and post-natal toxicity. The study conducted with Uvinul A Plus at 100, 300 and 1000 mg/kg/day showed that there were no toxic effects at the LD but effects were seen at the MD and HD. In the F0 parents, clinical signs of some of the animals in the MD and HD groups included symptoms such as urine-smeared fur and reduced nutritional state. The study investigators interpreted these signs as symptoms of poor health. In the HD group, signs of parental toxicity included lower food consumption and body weight gains. Lower body weight gains were also seen in the LD and MD male groups (by 5%) but the changes were not dose-dependent although statistical significance was seen in the MD group at week 17 ie. after the mating period. At necropsy, renal changes (chronic nephropathy and necrosis of renal papilla) were seen in three HD females although the study report did not consider these changes as treatment-related. Increased liver weights were seen in HD males but there were no treatment-related histological changes.

In the HD group of F0 parents, decreased implantation and higher post-implantation loss were seen. Post-implantation loss was also slightly higher (higher than concurrent and historical controls) in the MD group but the change was not statistically significant. The poor health seen in some maternal animals was reported to result in insufficient maternal care and cannibalisation of some pups by their mothers. Most of the effects (changes in clinical signs, food consumption, body weight gain, implantation) seen in F0 parents were also seen in the next generation adults (F1 parents). Statistically significant lower food consumption and body weights were also seen in the LD and MD groups of F1 adults but the changes were slight (< 10%) and did not show any dose-response relationship between these groups.

In the F1 pups, changes were seen only in the HD group. Decreased pup viability, lower body weight gain, delayed sexual maturation and decreased spleen weight were seen. Lower pup survival, body weight gains and spleen weight were also seen in the next generation (F2) pups. Some of the pup deaths were due to cannibalisation by their mothers.

In F1 parents, higher kidney weights were seen in males of the HD group. Immuno-histochemical examination of the renal tissue in males revealed higher amounts of α2u-globulin in the MD and HD groups. According to the study report, α2u-globulin in kidneys is a species-specific phenomenon in rats (males) and has no relevance to humans.

Based on the effects observed in two generations, the NOEL of Uvinul A Plus for reproductive toxicity is determined by the OTCMS evaluator to be 100 mg/kg/day (LD) since effects were seen at higher doses (300 and 1000 mg/kg/day) in parental animals as well as in their offspring. However, according to the study report, the NOAEL (no observable adverse effect level) for fertility and general reproductive performance was 300 mg/kg/day for the F0 and F1 parental rats and the NOAEL for overall general toxicity of the test substance was reported to be 100 mg/kg/day. The study report determined the overall NOAEL for developmental toxicity (growth and development of the offspring) to be 100 mg/kg/day which is identical to the NOEL determined by the OTCMS evaluator.

In summary, the studies submitted so far on reproductive toxicity in rats (a developmental toxicity study before and currently a 2-generation reproductive toxicity study) showed that

the overall NOEL for reproductive toxicity was 40 mg/kg/day (as determined before for the developmental toxicity study).

3.5 Genotoxicity

A series of *in vitro* studies and an *in vivo* study in mice (micronucleus study) previously showed that Uvinul A Plus was not mutagenic or clastogenic. The sponsor has now provided a mammalian cell mutation assay in mouse lymphoma L5178Y cells (TK+/-) which confirmed that there is no concern regarding genotoxicity.

3.6 Carcinogenicity

Concerns regarding carcinogenicity have been addressed before. Although a carcinogenicity study has not been provided by the sponsor, the sponsor was previously requested to provide an *in vivo* genotoxicity study which would give greater degree of assurance that the compound does not have the potential to be a genotoxic carcinogen. The sponsor provided an *in vivo* mouse micronucleus study which was assessed in the previous evaluation report. The negative results of that study as well as the current *in vitro* mutation test in mammalian cells have added to the negative results already seen in a series of genotoxicity assays assessing different end points confirming a low likelihood of genotoxic activity.

3.7 NOEL and margin of exposure (safety margin)

In toxicity studies the NOELs relevant for estimating the margin of exposure are: 1350 mg/kg/day in a 13-week oral toxicity study, 100 mg/kg/day in a 2-generation reproductive toxicity study and 40 mg/kg/day in a developmental toxicity study (all the studies were conducted in rats with oral administration of Uvinul A Plus). Hence the overall NOEL in toxicity studies was 40 mg/kg/day po.

In *in vivo* studies in rats, oral and dermal absorption of Uvinul A Plus were ~ 40% and ~ 3%, respectively (dermal absorption was 13.3 times lower than oral absorption). Based on this, the equivalent NOEL for the dermal route is estimated to be 40 mg/kg/day (oral NOEL) x 40% ÷ 3% = 533 mg/kg/day. Since dermal absorption in human skin is ~ 20 times lower than that in rat skin, the equivalent dermal NOEL for humans is estimated to be 533 x 20 = 10660 mg/kg/day.

For a sunscreen product containing 10% of Uvinul A Plus, it is assumed that 18 g of the product will be applied every day. Hence for a person weighing 50 kg, the amount applied per day is 18000 mg product x 10% Uvinul A Plus ÷ 50 kg bw = 36 mg/kg bw/day of Uvinul A Plus.

Since 36 mg/kg/day of Uvinul A Plus is applied topically, the safety margin at the intended dose (in comparison to the estimated dermal NOEL in humans) is 10660 mg/kg/day ÷ 36 mg/kg/day = 296. This safety margin is considered adequate.

It is noted that the SCCP (April 2008) has calculated a safety margin of 2222 for Uvinul A Plus which is 7.5 times higher than the safety margin calculated by the OTCMS evaluator. The difference is mainly due to the fact that the SCCP has used 200 mg/kg/day as the NOAEL for Uvinul A Plus whereas the OTCMS evaluator has used a NOEL of 40 mg/kg/day for the risk assessment. The SCCP has used a value of 36 g/day for the product applied on

the skin (whereas the OTCMS has used a value of 18 g/day) since according to the SCCP, in a worst-case scenario the consumer would use a "set of cosmetic products containing the same substance" and hence the SCCP has used a value of 17.79 g/day as suggested by the SCCNFP and an additional 18 g.

4 SUMMARY AND RECOMMENDATION

Diethylamino hydroxybenzoyl hexyl benzoate ("Uvinul A Plus") is a new UVA filter intended for use in listed sunscreen products at a concentration of 10%.

The MEC have considered the sponsor's application before in two meetings in the year 2006 and commented that there were still issues which needed to be addressed prior to approval of this substance. The pending issues were related to the absence of adequate data on: dermal and oral absorption, skin irritation potential on repeated administration, photosensitisation, reproductive toxicity and genotoxicity of the filter.

The sponsor has now provided all the required studies.

In vivo absorption studies in rats showed that dermal and oral absorption of Uvinul A Plus were ~ 3% and ~ 40%, respectively. In *in vitro* skin absorption studies, absorption was found to be ~ 20 times lower in human skin when compared to that of rat skin. Based on this, the *in vivo* dermal absorption in humans is likely to be ~ 0.15%.

A repeated insult patch test in humans showed that 10% Uvinul A Plus was not a skin irritant or a skin sensitiser. The substance was not phototoxic or photosensitiser in guinea pigs at this concentration. These studies have addressed the concerns raised in the earlier evaluation reports regarding local tolerance.

In studies submitted previously, Uvinul A Plus was found to be of low acute oral toxicity in rats and the no-observed-effect level (NOEL) for repeat dose toxicity was 1350 mg/kg/day in a 13-week repeat dose oral toxicity study in rats.

A 2-generation reproductive toxicity study was submitted to address the lack of data on fertility and general reproductive performance, and pre- and post-natal toxicity (only a developmental toxicity was submitted previously). In the new study, signs of toxicity in parental animals as well as in reproductive parameters were seen at 200 and 1000 mg/kg/day PO. Signs in the parental animals of F0 and F1 generations included lower food consumption and body weight gain, poor health and renal changes. Lower implantation and higher post-implantation loss were also seen at these doses. In the pups (F1 and F2), lower survival, delayed development and lower spleen weight were seen at the high dose. The NOEL for reproductive toxicity was 100 mg/kg/day in this study. In a developmental toxicity study in rats evaluated before, the NOEL was 40 mg/kg/day (based on maternal toxicity and skeletal variations in fetuses at higher doses).

An *in vitro* mutation study in mammalian cells (mouse lymphoma L5178Y) as well as the previous studies on genotoxicity (*in vitro* and *in vivo*) showed that there is no concern regarding genotoxicity.

Concerns regarding carcinogenicity have been addressed previously.

Calculation of the safety margin showed that there is sufficient safety margin at the intended exposure (~ 300 when the intended exposure is compared to the lowest NOEL in toxicity studies).

Based on the above, the sponsor's application is **supported**.

The name of the substance is not on the list of Australian Approved Names for Substances. The sponsor should apply to the TGA to get the chemical name approved.

The committee's opinion is requested.

5 EVALUATION OF SUBMITTED TOXICITY DATA

5.1 Pharmacokinetics

5.1.1 Oral absorption

^{14}C-Uvinul A Plus. Study on the bioavailability in rats. Fabian E and Landsiedel R. Experimental Toxicology and Ecology, BASF SE, D-67056 Ludwigshafen/Rhein, Germany. Project No. 02B0496/046024. 7 August 2008. GLP/QA: Yes.

In a study to investigate the extent of oral absorption and excretion, Uvinul A Plus (unlabelled and ^{14}C-radiolabelled in olive oil; radioactivity: ~ 0.5-2 MBq per animal) was administered at a dose level of 100 mg/kg (gavage) to Crl:WI (Han) strain Wistar rats. The study was conducted as two experiments. In the first experiment ("with a repeat component"), bile duct was cannulated and then Uvinul A Plus was administered by gavage to the animals (4/sex) while the second experiment was conducted in animals with an indwelling catheter in the bile duct (the animals were supplied to the study laboratory with a catheter already in the bile duct; 4 animals/sex).

In both the experiments urine, feces and bile were collected for 72 hours and total radioactivity was measured in each of the samples collected. In the second experiment, one female had insufficient bile flow and was excluded from data analysis.

The total number of animals used in the study is not clear. On page 16 of the study report, the number of animals has been stated as 24: "experiment with 8 animals; repetition of the experiment with additional 8 animals and confirmation experiment with 8 animals, bile duct catheterized by the supplier". Although the study has reported to use 24 animals, individual animal data were provided for only 15 animals (animal nos. 9-21, 23 and 24). The reason for the missing individual animal data (for animal nos. 1 to 8 and no. 22) is not clear.

The results are summarised in the table below. As seen in the table, oral absorption of Uvinul A Plus radioactivity was higher in females (% absorption in 72 h, in the two experiments: ~ 24% in males and 54% in females). Total recovery of the radioactivity was ~ 80-88%.

% Radioactivity in 72 h in different samples

Samples	**% of administered radioactivity**			
	Experiment I		**Experiment II**	
	Males	**Females**	**Males**	**Females**
Bile	11.28	19.76	10.78	23.59
Urine	13.79	22.62	10.12	35.24
Carcass	0.46	1.26	0.18	2.42
Cage washings	0.71	1.46	0.36	0.88
Absorption of radioactivity (total of the above)	**26.24**	**45.10**	**21.44**	**62.13**
Stomach and intestine	2.12	10.17	0.08	1.43
Feces	51.47	32.78	63.35	23.65
Total recovery of radioactivity	79.84	88.05	84.86	87.21

n = 4/sex. In the second experiment, one female was excluded from data analysis because of "insufficient bile flow" (7.84%).

Note:

On Page 18 of the study report, the doses were reported as 5 mg/kg bw for the experiments whereas in all other places of the report, the dose of Uvinul A Plus is reported as 100 mg/kg/day. It appears that the dose '5 mg/kg' refers to the radioactivity dose of ~ 5 MBq/kg or to the dose volume (5 mL/kg).

5.1.2 Dermal absorption (*in vivo* and *in vitro*)

Study on the dermal penetration of ^{14}C-Uvinul A Plus in rats. Fabian E and Landsiedel R. Experimental Toxicology and Ecology, BASF SE, D-67056 Ludwigshafen/Rhein, Germany. Project No. 01B0496/046023. 5 May 2008. GLP/QA: Yes.

In an *in vivo* study to investigate the extent of dermal absorption and excretion, a formulation (AS 675) containing 10% Uvinul A Plus (with added ^{14}C-Uvinul A Plus; specific activity of a.i.: 6.78 MBq/mg) was administered to 3 groups of male Crl:WI (Han) strain Wistar rats (4/group) at a nominal dose level (presumably of the non-radiolabelled substance) of 0.81 mg/cm^2 (~ 8.1 mg/animal; stated as "2.6 mg/kg" but presumably 26 mg/kg for rats weighing ~ 300 g). The test substance was applied at a volume of 100 µL over an area (10 cm^2) of skin (previously clipped) and covered with a permeable gauze and a semi-occlusive adhesive bandage for 12 hours. Test site skin was washed in all animals at the end of the exposure period. The first group was sacrificed immediately after the 12 h exposure period. The second and third groups were sacrificed at 24 and 120 hours, respectively (skin was washed again just before sacrifice). Samples of urine and faeces were collected during the treatment period. Total radioactivity was measured in the excreta, blood (blood cells and plasma), application site and surrounding area, carcass and skin washes.

After dermal application of 10% Uvinul A Plus, dermal absorption occurred within 12 hours since total absorption at 24 and 120 hours was comparable to that at 12 hours. Absorption was ~3% of the administered dose. Detectable levels of radioactivity were not seen in plasma.

Dermal absorption of radiolabelled Uvinul A Plus

	% of administered radioactivity		
Sacrifice time	12 h	24 h	120 h
Urine	0.08	0.28	0.76
Feces	0.02	0.07	0.79
Cage wash	0.06	0.11	0.17
Blood cells	0.04	0.04	0.03
Plasma	0.00	0.00	0.00
Carcass	2.76	1.82	1.41
Absorption of radioactivity (total of the above)	**2.96**	**2.31**	**3.16**
Surrounding skin	0.22	0.45	0.13
Protective cover[#]	0.32	6.88	5.45
Application site	4.36	1.36	0.71
Initial skin wash	97.13	100.34	107.34
Second skin wash	-	0.97	0.09
Total recovery	104.99	112.30	116.88

n = 4/sacrifice time point. Dose of Uvinul A Plus: 0.81 mg/cm^2 (dermally; area: 10 cm^2), exposure for 12 h.
[#]The application site was covered semi-occlusively.

In vitro penetration and permeation study of Uvinul A Plus (test substance no. 04/0496) in human skin. Kaca M. Across Barriers GmbH, Science Park 1, 66123 Saabruecken, Germany. Report No. C-10105-039-0308. Project No. BASF: 52H0496/049111. 8 August 2008. GLP/QA: Yes.

The *in vitro* absorption of Uvinul A Plus from a formulation (K9081 ointment containing 10% Uvinul A Plus, radiolabelled; full composition of K9081 not provided) across human skin from 3 female donors was examined. The test formulation was applied to prepared skin membranes which were positioned across a chamber (Franz diffusion cells; diffusion area: 1.8 cm^2) that contained a receptor fluid. The formulation was applied at ~ 18 mg (containing 1.8 mg of Uvinul A Plus) and left in contact for a period of 24 hours at 32 ^{0}C.

Samples of the receptor fluid were collected at 0, 2, 4, 6, 8, 20, 22 and 24 hours after application of the test substance. At the end of the experiment, the skin biopsies were separated into stratum corneum and deeper layers by tape stripping and distribution of Uvinul A Plus was monitored in different skin layers, including that remaining on the surface of the skin. The concentration of test substance in the receptor fluids, different layers of the skin, and in the donor and receptor washings was analysed by liquid scintillation counting (LSC). Twenty strips (layers) of stratum corneum were analysed. Out of these, the top two strips were analysed separately due to potential contamination by residual test substance on the surface of the skin. Caffeine (aqueous solution, 10 mg/mL; 1.2 mL; radiolabelled) was used as a reference compound.

In vitro dermal absorption of Uvinul A Plus in 24 hours

	Amount of Uvinul A Plus (mean ± SD)	
	µg	***% of applied dose***
Skin wash	1784.66 ± 189.60	*99.00 ± 9.60*
2 Tape strips (Stratum corneum)	27.88 ± 20.84	*1.54 ± 1.12*
18 Tape strips (Stratum corneum)	7.42 ± 5.58	*0.41 ± 0.30*
Remaining skin (deeper skin)	1.96 ± 0.98	*0.11 ± 0.05*
Receptor compartment	0.31 ± 0.25	*0.02 ± 0.01*
Total recovery	-	*101.08 ± 10.06*

Abdominal skin samples from 3 female donors were used. Dose applied: 1801.31 ± 41.08 µg. The results shown are the mean values of two experiments (the first experiment conducted in duplicate; the second in duplicate for one sample, and single for two samples).

The results showed that the 24-hour cumulative absorption of the formulation containing 10% Uvinul A Plus across the skin was 0.13% excluding all stratum corneum and 0.54% including lower layers of stratum corneum (excluding the top 2 layers which were likely to be contaminated with residual test substance on the surface of the skin). For caffeine, the extent of absorption (%) was not stated but the mean permeability coefficient (P_{app}) was ~ 2.5 E-08 cm/s (n = 3) which was comparable to the values obtained previously in other human skin specimens.

Note:

Three Franz cells which did not meet the limits of the requirements of SCCP (acceptable total recovery: 100 ± 15%), were not included by the study investigators in the data analysis. The recovery was 125.3%, 80.0% and 84.4% in these cells.

In vitro penetration and permeation study of Uvinul A Plus (test substance no. 04/0496) in rat skin. Kaca M. Across Barriers GmbH, Science Park 1, 66123 Saabruecken, Germany. Report No. C-10105-039-0308. Project No. BASF: 50H0496/049112. 8 August 2008. GLP/QA: Yes.

The *in vitro* absorption of Uvinul A Plus from a formulation (K9081 ointment containing 10% Uvinul A Plus, radiolabelled; full composition of K9081 not provided) across rat skin (from 7 female rats, strain not stated) was examined. The test formulation was applied to prepared skin membranes which were positioned across a chamber (Franz diffusion cells; diffusion area: 1.8 cm^2) that contained a receptor fluid. The formulation was applied at ~ 18 mg (containing 1.8 mg of Uvinul A Plus) and left in contact for a period of 24 hours at 32 ^{0}C.

The extent of absorption of the test substance was determined by removing samples of the receptor fluid at 0, 2, 4, 6, 8, 20, 22 and 24 hours after application of the test substance. At the end of the experiment the skin samples were separated into stratum corneum and deeper layers by tape stripping and distribution of Uvinul A Plus was monitored in different skin layers, including that remaining on the surface of the skin. The concentration of test substance in the receptor fluids, different layers of the skin, and in the donor and receptor washings was analysed by LSC. Eight strips (layers) of stratum corneum were analysed. Out of these, the top two strips were analysed separately due to potential contamination by residual test substance on the surface of the skin. Caffeine (aqueous solution, 10 mg/mL; 1.2 mL; radiolabelled) was used as a reference compound.

In total, 10 skin samples from 7 rats were assayed in two experiments (the first experiment conducted in duplicate in 3 membranes; the second once in 4 membranes). Only 4 out of 10 samples (4 out of 10 Franz cells with skin from 4 donors) fulfilled the quality criterion. According to the study investigators, the deviations of total recovery from the quality criteria mainly stemmed from the test substance recovered with the skin wash (see table below).

In vitro dermal absorption of Uvinul A Plus in 24 hours

	Amount of Uvinul A Plus (mean ± SD)			
	All skin membranes		***'Qualified skin membranes'#***	
	µg	**% of applied dose**	**µg**	***% of applied dose***
Skin wash	1873.6 ± 658.5	102.9 ± 34.9	1388.4 ± 278.7	*78.0 ± 14.9*
2 Tape strips (Stratum corneum)	159.1 ± 242.9	8.76 ± 13.17	149.7 ± 85.9	*8.46 ± 4.95*
6 Tape strips (Stratum corneum)	104.8 ± 115.2	5.86 ± 6.47	134.8 ± 98.1	*7.65 ± 5.66*
Remaining skin (deeper skin)	104.8 ± 195.2	5.80 ± 10.78	46.8 ± 36.4	*2.65 ± 2.10*
Receptor compartment	0.86 ± 0.26	0.05 ± 0.01	0.74 ± 0.27	*0.04 ± 0.02*
Total recovery	-	*123.4 ± 32.2*	-	*96.8 ± 4.6*

Dose applied: 1804.9 ± 31.6 µg or 1777.3 ± 30.5 µg (for all skins and qualified skin, respectively). Skin samples from 7 female rats were used. The results shown are the mean values of two experiments (the first experiment conducted in duplicate in 3 membranes; the second in single in 4 membranes).
#Excluding membranes which showed recovery outside the limit of 100 ± 15%.

The results (in the 'qualified' skin membranes which showed total recovery of 100 ± 15%) showed that the 24-hour cumulative absorption of 10% Uvinul A Plus across the skin was 2.69% excluding all stratum corneum and 10.3% including lower layers of stratum corneum (excluding the top 2 layers which were likely to be contaminated with residual test substance on the surface of the skin). For caffeine, the extent of absorption (%) was not stated but the mean permeability coefficient (P_{app}) was ~8.2 E-08 cm/s (n = 7) which was double the values obtained previously in human skin specimens (~ 4.3 E-08 cm/s, n = 16; previous values for rat skin were not provided).

5.2 Local tolerance

5.2.1 Skin sensitisation

100 Human subject repeat insult patch test skin irritation/sensitization evaluation (semi-occlusive patch). Cantor Research Laboratories, Inc. 630 Route 303, Blauvelt, NY 10913, USA. CR Ref. No. RIPT.E1217-B1.SO.100.DERM.BAS. 3 April 2008. QA/GCP: Not stated.

In a repeated insult patch test (RIPT) conducted in humans (nine 24 h semi-occlusive applications; n = 101) with a formulation containing 10% Uvinul A Plus (o/w emulsion), there were no skin reactions during the induction (skin reactions were monitored at 48 or 72 hours after patch application) or challenge phase (skin reactions were monitored up to 48 hours after application). The test substance was not a skin irritant or a sensitiser.

In a separate study report, CR Ref. No. RIPT.E1217-B1.SO.100.DERM.BAS (dated 3 April 2008), the above formulation, minus the active ingredient (Uvinul A Plus), was found to be non-irritant and a non-sensitiser in the same subjects.

5.2.2 Photoirritation and photosensitisation

Photoirritation and photosensitization by cutaneous route in guinea pigs. Centre International de Toxicologie (Citrus junos seed extract), Miserey - BP 563, 27005, Evreux Cedex, France. Rokh N. BASF Project No. 48H0636/029038. 4 July 2008. GLP/QA-yes.

This study assessed the photo-allergic potential of Uvinul A Plus in a cutaneous sensitisation assay using 3 groups of Hartley Crl: (HA; COBS-VAF) BR guinea pigs (5/sex for groups 1 and 3; 10/sex for group 2):

Group	n	Induction phase		Challenge application	
		Anterior left flank	Anterior left flank	Posterior left flank	Posterior left flank
1	10	Test substance	-	Test substance	-
2	20	Test substance + UV	Vehicle + UV	Test substance + UV	Vehicle + UV
3	10	Vehicle + UV	UV	Test substance + UV	UV

Induction phase: 6 applications (days 1-8). Challenge application on day 22. - None.

During the induction phase, six topical applications of 0.1 mL Uvinul A Plus (10% in acetone; open application followed by a gentle massage) or vehicle were applied over a period of 8 days to a previously shaved area of skin, ~ 4 cm^2 in size. Approximately 30 min after each application, both anterior flanks of the animals of Groups 2 and 3 were irradiated with UV (A and B; 'infra-erythematogenic'). The irradiation was 'infra-erythematogenic' (score of erythema ≤0.5), the dose equating to ~ 9 Joules/cm^2 of UVA and 0.08 Joule/cm^2 of UVB. The non-irradiated parts were protected from the UV rays. Cutaneous reactions were scored at 1, 4 and 24 hours after the first application and ~ 24 hours after the subsequent applications. After the last induction application, the animals were not treated or irradiated for 13 days. On day 22, the animals were treated (naive sites; posterior flanks) with Uvinul A Plus and UV irradiation (30 minutes after Uvinul A Plus; the non-irradiated regions were protected with a cardboard mask) as shown in the table. Skin reactions were monitored at 1, 4, 24 and 48 hours after the challenge application.

In this study, the concentration of the test substance (10%) were selected from a preliminary study in which guinea pigs were tested with 5-50% Uvinul A Plus. The maximum non-irritant concentration was 10%.

In the main study, comparable skin reactions were seen at the vehicle and treated sites (with or without UV irradiation), with mean severity ≤1.0 in both the groups (most of the reactions were with a score of nil or 0.5, questionable erythema; a few discrete or patchy areas of erythema were also seen, score = 1). Uvinul A Plus (10%) was not a photoirritant or a photosensitiser in this study.

Non-concurrent positive controls from the study laboratory (conducted 1-2 years prior to the study date; 8-methoxypsoralen 0.1% in corn oil; "phenothiazine" 1-100% during induction and 100% at challenge) showed that the animal model was appropriate to detect photosensitisation.

5.3 Reproductive toxicity

Uvinul A Plus. Two-generation reproduction toxicity study in Wistar rats. Continuous dietary administration. Schneider S et al. BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project ID: 70R0636/02075. 20 November 2006. GLP/QA: Yes.

A 2-generation reproduction study with dietary administration of Uvinul A Plus (purity ≥98.8%) was performed in Wistar Crl:WI(Han) rats (F0 parental generation; 25/sex). Uvinul A Plus was administered at target dose levels of 0, 100, 300 and 1,000 mg/kg bw/day. After at least 75 days of initial treatment, F0 males and females were mated to produce F1 litters. Groups of 25/sex (except in the control group: one female was excluded due to a technical error) selected from F1 pups were retained as the F1 parental generation and were mated to produce F2 litters. Uvinul A Plus was administered continuously throughout the two

generations. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals (F0 parents were sacrificed after the weaning of F1 pups). The effects on mortality, clinical signs, body weight, food consumption, oestrous cycle, mating, gestation and delivery parameters, and pup development were examined.

All pups were examined macroscopically at necropsy and the weights of brain, spleen and thymus in one pup/sex/litter were measured. Sexual maturation (day of balanopreputial separation/vaginal opening) of all pups selected to become F1 parental generation animals was determined. Sperm parameters (motility in all groups; sperm count and morphology in control and HD groups only) were assessed in F0 and F1 generation males at scheduled sacrifice.

All F0 and F1 parental animals were assessed for changes in organ weights, gross pathology and histology with special attention paid to the organs of the reproductive system. A quantitative assessment of primordial and growing follicles in the ovaries was performed for all control and HD F1 parental females. Furthermore, the livers of all F0 parental animals and the kidneys of all F1 male parental animals were assessed histologically.

The day on which sperm was detected in a vaginal smear was denoted "day 0 p.c." (post coitum) and the following day as "day 1 p.c.". Similarly, the day after delivery was denoted as "day 1 p.p." (post-partum).

Functional observations (eg. motor activity, sensory function etc.) were not monitored in the offspring.

Analysis of the food consumption showed that the mean actual achieved doses of Uvinul A Plus were comparable to the intended doses (see table below).

Actual achieved doses of Uvinul A Plus (mg/kg/day; mean)

	LD	**MD**	**HD**
F0 Males	101	304	1010
F0 females (premating)	104	311	1033
F0 females - gestation period - lactation period[#]	 103 99	 312 294	 928 952
F1 Males	101	303	1010
F1 females (premating)	102	306	1017
F1 females - gestation period - lactation period[#]	 101 94	 302 281	 955 903

Nominal doses: 100, 300 and 1000 mg/kg/day for the LD, MD and HD groups, respectively.
[#]Food consumption was recorded for days 1-14 post partum only.

F0 parents

Clinical signs, mortality, body weight and food consumption

One HD female died during week 18. The cause of death (no clinical signs) could not be established at necropsy. In two MD females, seven HD females and three HD males, urine-smeared fur was seen (starting from the pre-mating/mating period) which was interpreted by the study investigators as an indication of impaired general state of the animals caused by treatment. There were no treatment-related effects on the duration of the oestrous cycle

(mean duration of the cycle: 3.9, 4.1, 3.9 and 4.3 days in the control, LD, MD and HD groups, respectively).

During gestation, a "poor general state", "reduced nutritional state" and piloerection were seen in 3 HD females. Another HD female showed a "reduced nutritional state" shortly before it delivered (day 21 p.c.). The fur of 4 HD females was smeared with urine around the anogenital region.

During the first few days of lactation, poor general state, reduced nutritional state and piloerection were still recorded in one HD female. All pups of this female died *in utero* or were stillborn. Another HD female, which showed a reduced nutritional state on lactation days 0-11, delivered just one pup which was cannibalized by its mother on the same day. A total litter loss was recorded in another HD female. This dam reportedly neglected nursing its litter properly. All its pups died or were cannibalized on or before day 2 p.p. Urine-smeared fur (around anogenital region) persisted in a HD female up to lactation day 10.

Mean food consumption and body weights were lower in the HD group. In HD males, the food consumption was significantly reduced throughout the treatment period and the overall intake during weeks 0-17 was lower by 11%. In HD females, food consumption was significantly lower by 12%, 28% and 22%, during the premating, gestation and lactation periods, respectively.

In HD males, body weights and weight gains were significantly reduced during the treatment period (body weight at week 17 was lower by ~ 14%; overall weight gain lower by ~ 22%). In the LD and MD males, body weights were slightly lower (by ~ 5%; similar effects in both the groups). The changes in these two groups were not statistically significant except at week 17 in the MD group (overall weight gain lower by ~ 7%). In HD females, body weights and weight gains were significantly lower during the premating, gestation and lactation periods (bw by 11%, 24% and 10%, respectively).

Reproduction and development parameters

Mating index (100% in all groups) or fertility index (92% in the control and 96% in the treated groups) was not affected by treatment. In the HD group, the mean number of implantation was significantly lower (by 17%; mean number of implants: 11.6, 11.1, 10.7 and 9.6 in the control, LD, MD and HD groups, respectively). In this group, 3 HD dams did not deliver pups (but had dead implants *in utero*) and consequently the postimplantation loss was significantly higher (22.1% vs 4.4% in the controls). The gestation index was lower in the HD group (83% vs 100% in the other groups). The mean number of pups delivered was significantly lower in the HD group (11.0, 10.5, 9.8 and 8.8 in the control, LD, MD and HD groups, respectively). The live birth index was not significantly affected by treatment. Although a lower number of implants and pups (see above for the incidence) were seen in the MD group, the incidences were within historical control range. However, the postimplantation loss in this group was higher and outside the historical control range although not statistically significant (% postimplantation loss: 4.4, 5.3, 9.1 and 22.1 in the control, LD, MD and HD groups, respectively; historical control range: 2.5-8.4%).

Necropsy of F0 parents

Absolute and relative liver weights were significantly higher (by 11% and 30%, respectively; bw was lower by 15%) in the HD male group. There were no treatment-related histological changes in the liver (all animals were examined). The right kidney was enlarged and light brown in one HD female, and discolouration of kidneys was seen in 2 HD females. Histological changes in kidneys included chronic nephropathy and necrosis of renal papilla in 3 HD females (nil in other groups but only 0, 1, 1 and 3 animals were examined histologically for renal changes in the control, LD, MD and HD groups, respectively). However, the study report considered the histological changes in kidneys as incidental.

Sperm parameters were not affected by treatment.

F1 pups (up to weaning)

Clinical signs, mortality, body weight, sexual maturation, organ weights and gross pathology

There were no treatment-related changes in clinical signs. Pup viability was significantly lower in the HD group during days 0-4 p.p. (viability index was 95% in this group; 99% or 100% in other groups) but survival during days 4-21 p.p. was not affected (lactation index was 99% in the control group, 100% in the treated groups). The number of males was slightly lower in the HD group (males: 42% in the HD group; 49% or 50% in the other groups). Pup weights (males and females combined; comparable values in males and females) on day 1 p.p. was 14% lower in the HD group (statistically significant). Pup weight gain was significantly lower in this group throughout the lactation period. At weaning, pup weights in the HD group were 24% below the control group (significant). Body weights or weight gains were not affected in the LD and MD groups.

In the HD group, delayed (statistically significant) vaginal opening in females (mean age for vaginal opening: 31.6, 31.6, 32.0 and 33.2 days in the control, LD, MD and HD groups, respectively) and preputial separation in males (mean age: 43.4, 43.4, 43.2 and 44.3 days in the control, LD, MD and HD groups, respectively) were seen. According to the study report, these changes were related to significantly lower body weights in the HD group (by 9% and 14% in females and males, respectively; at the time of vaginal opening and preputial separation, respectively).

There were no treatment-related gross pathological changes at necropsy. In the HD group, absolute and relative spleen weights were significantly lower in males and females (by 45% and 28%, respectively; males and females combined). This effect appears to be treatment-related since the relative weight was decreased although body weights were lower (the study report interpreted the change as 'presumably secondary to lower pup weights/body weight gains in this group). Changes in brain and thymus weights were seen but these appear to be related to lower body weights.

F1 parents

Clinical signs, mortality, body weight and food consumption

There were no deaths. After mating, three sperm positive control females, one LD female and three HD females did not deliver any F2 pups. Urine-smeared fur around the anogenital region occurred in two HD females (study week 10 or 11, mating period; gestation days 0-3; first few days of lactation). One of these animals also showed a "reduced nutritional state" on

gestation day 20 (shortly before delivery) and during the first few days of lactation. This animal cannibalized all its pups on day 1 p.p. Another HD female showed a poor general state, a reduced nutritional state, piloerection and insufficient maternal care of pups during lactation days 0-6. All pups of this animal were stillborn/dead on day 1 p.p.

A total litter loss was also recorded for a HD dam on day 3 p.p. ("all pups died/were cannibalized by their mother"). Insufficient maternal care of pups was reported for another HD female on lactation days 0-1, but nearly all of its pups survived. All pups of a MD dam were stillborn.

In the HD group, statistically significant lower food consumption was seen during the entire treatment period in males (overall consumption lower by 17%) and females (by 15%, 27% and 24% during the premating, gestation and lactation periods, respectively). In the LD and MD male groups, statistically significant decrease in food consumption was seen at some time points. The overall food consumption in these groups was slightly lower without showing dose-response relationship (consumption lower by 5% and 4% in the LD and MD groups, respectively; not affected in females).

Throughout the treatment period, body weight gain was lower in HD males and females. In HD males, the mean final body weight was 24% lower (significant). In HD females, the weights were significantly lower by 16%, 25% and 17% at the end of premating, gestation and lactation periods, respectively. As with food consumption, body weights in LD and MD males were slightly but significantly lower (final weights by 6% and 8%, respectively).

There were no treatment-related effects on the duration of the oestrous cycle (mean duration: 3.9-4.0 days) or on sperm parameters.

Reproduction and development parameters

Mating (100% in all groups) and fertility indices (88%, 96%, 100% or 88% in the control, LD, MD and HD groups, respectively; 3, 1, 0 and 3 females in the control, LD, MD and HD females were not pregnant; n = 24 or 25) were not affected by treatment. There was no treatment-related effect on the onset of mating (mean duration until sperm was detected: 2.6, 2.6, 2.2 and 2.4 days in the control, LD, MD and HD groups, respectively; values were reported to be within historical control range). The duration of gestation was slightly higher in the HD group, but the change was not statistically significant.

In the HD group, the mean number of implantations was lower (by 13%; mean number: 10.8, 11.3, 11.2 and 9.4 in the control, LD, MD and HD groups, respectively; statistically non-significant but outside the historical control range of 10.2-11.7 implants/dam). Post-implantation loss did not show any dose-dependent effect (% loss: 7.2, 2.8, 4.2 and 6.4 in the control, LD, MD and HD groups, respectively).

The gestation index was 100%. The mean number of pups delivered in the HD group was lower and outside the historical control range although the change was not statistically significant (10.1, 10.9, 10.7 and 8.8 in the control, LD, MD and HD groups, respectively; historical control range: 9.3-11.7). The live birth index or the number of stillborn pups was not significantly affected by treatment.

Necropsy of F1 parents

Absolute and relative kidney weights were significantly higher in the HD male group (by 17% and 64%, respectively; bw was lower by 25%).

Gross pathological changes were not remarkable.

Eosinophilic droplets (immunohistochemical examination revealed α2u-globulin, a male-specific protein in rats) were observed in some of the tubular epithelial cells in all males, including controls but the amount of α2u-globulin was higher in the MD and HD groups (see table below).

Incidence of eosinophilic droplets in males

Grade for α2u-globulin				
Control		**LD**	**MD**	**HD**
Grade 1	14	12	3	-
Grade 2	11	13	19	17
Grade 3	-	-	3	8

n = 25

At the differential ovarian follicle count (primordial and growing follicles), the primordial count (and the total count: primordial+growing follicles) was found to be lower in the HD group (by 14%) although the changes were not statistically significant.

Chronic nephropathy and necrosis of renal papilla were seen in one HD female (only this animal was examined for renal changes in females; chronic nephropathy was also seen in control and treated male groups).

Sperm parameters were not affected by treatment.

F2 pups (up to weaning)

Clinical signs, mortality, body weight, sexual maturation, organ weights and gross pathology

Pup mortality was significantly higher at the HD: the viability index (survival) for days 0-4 was 99%, 98%, 100% and 85% for the control, LD, MD and HD groups, respectively. The lactation index (survival) for days 4-21 was also lower in the HD group (97% vs 100% in the other groups; statistically significant). Some of the deaths were caused by maternal cannibalisation.

Sex ratio was not affected by treatment.

Pup weight gain was significantly lower in the HD group (males as well as females) throughout the lactation period. On day 1 p.p., body weights were 18% lower in this group (males and females combined) and on day 21 p.p., the weights were lower by 26%. In the MD group also the weights were slightly lower on day 21 (by 6%, statistically significant).

Organ weight analysis (only brain, thymus and spleen) revealed significantly lower spleen weights in males and female pups of the HD group (absolute and relative weights by 46% and 28%, respectively; males and females combined). According to the study report, all values were within historical control ranges. For spleen, the historical control ranges (males and females combined; comparable values in males and females) showed high variation: 0.027-

0.392 g for the absolute weights (mean: 0.219 g; n = 513 litters) and 0.136-0.741 for the relative weights (organ to body weight ratio; mean: 0.467). In the HD group, the mean spleen weights were 0.132 g and 0.355 for the absolute and relative weights, respectively. Based on the mean values, and also that similar changes were seen in the F1 pups (absolute and relative spleen weights were lower by 45% and 28%, respectively), the OTCMS evaluator considers the changes in spleen weights of the HD group as treatment-related. Changes in brain and thymus appear to be related to lower body weights.

Necropsy examination of the pups (n = 177-266 pups/group) revealed no treatment-related abnormality (the incidences were comparable to the control group or historical controls).

The NOEL for this study is 100 mg/kg/day (LD) for parental toxicity as well as reproductive toxicity.

5.4 Genotoxicity

Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with Uvinul A Plus. Wollny HE. RCC, Cytotest Cell Research GmbH, In den Leppsteinswiesen 19, D-64380 Rossdorf. RCC-CCR Study Number 1046900. 28 December 2006.

In an *in vitro* mammalian cell mutation assay (two experiments), Uvinul A Plus (vehicle: DMSO) was tested in mouse lymphoma L5178Y cells (thymidine kinase locus, TK+/-) at up to 62.5 or 15.0 µg/mL in the absence of S9 mix (in the first and second experiments, respectively) or up to 250 or 100 µg/mL in the presence of S9 mix (in the first and second experiments, respectively). The treatment period was 4 hours in the first experiment (± S9) and 4 or 24 hours in the second experiment (with and without metabolic activation, respectively). Higher concentrations could not be tested because of cytotoxicity and turbidity or precipitation. Methyl methane sulphonate (-S9; 19.5 µg/mL) and cyclophosphamide (+ S9; 4.5 µg/mL) were used as positive controls.

When compared to the vehicle and historical controls, mutant frequency was higher (linear regression analysis showed significant concentration-dependent trend with the exception of the second culture: without metabolic activation in the first experiment and with metabolic activation in the second experiment) at a few concentrations (see bolded figures in the table below) but the changes were not concentration-dependent (as in the case of Experiment II with S9, Culture I) or consistent (occurrence in only one of the two cultures or in only one of the two experiments). The study report stated that all absolute values of the mutation frequency in both experiments remained within the historical control range. Based on these results, it is assessed that Uvinul A Plus was not mutagenic in this assay. The positive controls showed appropriate response.

Number of mutant colonies (mean)

Treatment	**Concentration µg/mL**	**S9**	**Mutant colonies/10^6 cells**	
			Culture I	**Culture II**
Experiment I (4 h treatment)				
Control (no treatment)			54	56

		-		
Control (DMSO)			112	97
Historical control (solvent)			*106*	*106*
Positive control (MMS)			358	351
Uvinul A Plus	3.9		107	**115**
	7.8		95	68
	15.6		94	99
	31.3 (p)		94	95
	62.5 (p)		**152**	97
Control (no treatment)		+	65	59
Control (DMSO)			44	61
Historical control (solvent)			*110*	*110*
Positive control (CPA)			346	324
Uvinul A Plus	15.6		65	57
	31.3 (p)		68	44
	62.5 (p)		67	90
	125.0 (p)		90	84
	250.0 (p)		75	83
Experiment II (24 h treatment)				
Control (no treatment)		-	98	56
Control (DMSO)			61	70
Historical control (solvent)			*107*	*107*
Positive control (MMS)			524	665
Uvinul A Plus	1.9		88	67
	3.8		51	65
	7.5		76	92
	10.0		71	107
	15.0		70	**115**
Experiment II (4 h treatment)				
Control (no treatment)		+	37	94
Control (DMSO)			70	165
Historical control (solvent)			*110*	*110*
Positive control (CPA)			658	661
Uvinul A Plus	3.0		**130**	97
	6.3		90	73
	12.5		102	134
	50.0		**134**	133
	100.0		**140**	156

Methyl methane sulphonate (MMS; 19.5 µg/mL) and cyclophosphamide (CPA; 4.5 µg/mL) were used as positive controls.

Attachment 1

Extract of MEC Minutes 6 April 2006

4.1 Diethylamino hydroxybenzoyl hexyl benzoate

This was an application by BASF Australia Pty Ltd for approval of diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus) for use as a UVA filter in listed sunscreen products and cosmetics at concentrations of up to 10%.

Diethylamino hydroxybenzoyl hexyl benzoate was approved by the European Union in January 2005 for use in sunscreen products at concentrations of up to 10%. That approval was based on an assessment by the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) of the European Commission.

The sponsor advised that diethylamino hydroxybenzoyl hexyl benzoate is "yet to be approved" for use in New Zealand, Canada, USA, UK or Sweden, and that, as it has not yet been marketed, no adverse events have been reported. The sponsor asserted that no adverse effects are known and none would be suspected, based on the studies presented.

Pharmaco/toxico-kinetics

An *in vitro* study reported an overall percutaneous absorption through pig epidermal membranes of ~1% of the test substance over 24 hours. The evaluator queried the reliability of the study, due to technical problems such as leaky membranes and high variability in the individual data, and had noted that the SCCNFP did not consider the study to be valid for similar reasons. A summary of a second *in vitro* dermal absorption study (that was submitted to the SCCNFP, but not to the TGA) reported a lower percutaneous absorption of 0.04%. The results of the two *in vitro* studies showed high variability, both within one study, and between the studies. The evaluator considered that the *in vitro* studies may not be reliable, and had recommended that an *in vivo* dermal absorption study should be provided. The evaluator had also recommended the provision of an oral absorption study, to allow assessment of exposure associated with the oral repeat dose and reproductive toxicity studies in rats.

No data were provided on the metabolism of diethylamino hydroxybenzoyl hexyl benzoate. The sponsor had indicated that specific ADME (absorption, distribution, metabolism and elimination) studies with radioactive material have not been performed, as the substance would have low toxicity or low absorption and metabolites were unlikely to be mutagenic or carcinogenic. The evaluator has not accepted the sponsor's arguments, but contended that there would be at least limited bioavailability after oral exposure, and noted that a developmental toxicity study suggested there is some oral absorption. Systemic toxicity may also occur following dermal exposure.

The SCCNFP had calculated a safety margin of 6667 (based on a maximum absorption through the skin of 0.1 µg/cm^2 and a NOAEL of 200 mg/kg in a rat developmental study). The TGA evaluator had calculated a lower safety margin of 111, assuming dermal absorption of ~1%, based on the *in vitro* dermal study (assessed to be unreliable) submitted to the TGA.

Interaction with other UV filters

No data were provided on possible interactions between diethylamino hydroxybenzoyl hexyl benzoate and other UV filters, and information on its stability was in isolation from other UV filters. The substance was resistant to degradation under extremes of pH and temperature for up to 3 months, and was not photodegraded significantly after up to 6 hours of UV irradiation.

Local tolerance

The TGA evaluator classified diethylamino hydroxybenzoyl hexyl benzoate as a slight eye irritant, based on data using rabbits, but had noted that eye irritation may not occur at the intended concentration of 10% in topical products.

Diethylamino hydroxybenzoyl hexyl benzoate was not a primary skin irritant after a single application in rabbits, and did not appear to be a skin irritant after a single application (at up to 25%) in guinea pigs. A concentration of 25% did not induce skin hypersensitivity in guinea pigs. While the substance did not show a sensitisation or photosensitisation response in studies in guinea pigs, the evaluator had questioned the validity of the photosensitisation study. The evaluator had concluded (based on information reported in the sponsor's Product Safety document about a repeat-dose dermal irritation study in guinea pigs that was not provided to the TGA or evaluated by the SCCNFP) that repeated application may cause slight skin irritation at the intended concentration of 10%.

No local tolerance studies in human subjects were provided. The evaluator had recommended that a repeated insult patch test in humans should be provided, to address concerns regarding the potential for skin irritation following repeated dermal exposure (as seen in guinea pigs).

Acute/repeat dose toxicity

An acute oral toxicity study in rats showed that diethylamino hydroxybenzoyl hexyl benzoate has low acute toxicity (>2 g/kg). The NOEL (no-effect level) in a 13-week repeat dose oral toxicity study in rats was determined to be the high dose (1350 mg/kg/day). The evaluator noted that the observed increases in liver weight and changes in plasma bilirubin, together with toxic effects seen in a developmental toxicity study in rats, suggested some degree of systemic exposure following oral dosing. The extent of oral bioavailability in the rat could not be estimated, as kinetic data were not provided.

Reproductive toxicity

The evaluator had determined a NOEL of 40 mg/kg/day (the low dose) in a developmental toxicity study in rats, based on maternal and foetal toxicity (skeletal variations) seen at the medium and high doses (200 and 1000 mg/kg/day). The sponsor did not consider these to be toxic effects, and had determined NOELs of 200 mg/kg/day for maternal toxicity and 1000 mg/kg/day for developmental toxicity. There was no assessment of possible oestrogenic activity.

Genotoxicity

Four *in vitro* genotoxicity assays produced negative results, indicating that diethylamino hydroxybenzoyl hexyl benzoate was not genotoxic *in vitro* in the presence or absence of UV

radiation. The evaluator had recommended that an *in vivo* genotoxicity study (mouse micronucleus assay) should also be submitted, to address concerns about the potential genotoxicity of metabolites of diethylamino hydroxybenzoyl hexyl benzoate.

Cytotoxicity

Diethylamino hydroxybenzoyl hexyl benzoate was cytotoxic *in vitro* at 50 and 100 µg/mL. Comparison of results for the test substance and untreated control showed no evidence of phototoxicity.

Carcinogenicity

No carcinogenicity studies were submitted but the sponsor provided an argument addressing potential carcinogenicity. The committee noted the discussion in the evaluation report of the sponsor's argument. The evaluator contended that, overall, the sponsor had not provided adequate justification for not providing a long-term carcinogenicity study, and had noted the following issues:

- The submitted *in vitro* dermal absorption study in pig skin was inconclusive, a second *in vitro* study (also using pig skin) was not provided to the TGA, and the evaluator had recommended that an *in vivo* dermal absorption study should be provided (refer to *Pharmaco/toxico-kinetics*, above);
- As there were no direct data on metabolism (ADME study) of diethylamino hydroxybenzoyl hexyl benzoate, the potential for the formation of active metabolites is not accurately known;
- A repeated dermal irritation study in guinea pigs was not submitted, the limited dermal irritation data indicated that a 10% concentration of the substance may produce skin irritation on repeated administration, and the evaluator had questioned the validity of the study results of a photoallergenicity study in guinea pigs;
- No clinical skin irritation or skin sensitisation studies (human) were provided;
- There could be a non-genotoxic mechanism for carcinogenesis involving chronic skin irritation and inflammation – the animal skin irritation studies indicated that, while diethylamino hydroxybenzoyl hexyl benzoate may not be an irritant at a concentration of 10% after a single administration, it could be an irritant on repeated dermal exposure;
- Toxicity studies were of short duration (up to 13 weeks) and may not detect carcinogenicity potential;
- Genotoxicity was not adequately addressed, as *in vivo* genotoxicity data were not provided;
- As the *in vitro* photomutagenicity or photoclastogenicity assays did not include metabolic activation, it is not known whether photogenotoxic metabolites are formed;
- Diethylamino hydroxybenzoyl hexyl benzoate was shown to be cytotoxic in a cytotoxicity assay and *in vitro* genotoxic assays; and
- Human experience with the substance is limited, given that it was only approved in Europe in January 2005.

Discussion

The evaluator had recommended that additional data should be provided before a decision can be made regarding this application:

- *In vivo* oral and dermal absorption studies (as the submitted *in vitro* dermal absorption study did not allow a reliable estimate of dermal absorption);
- An *in vivo* genotoxicity (mouse micronucleus) study;
- The two studies referred to in the sponsor's application that were not provided to the TGA (a second *in vitro* dermal absorption study using pig epidermal membrane and a repeated dose topical application study in guinea pigs);
- A repeated insult patch test in humans (to address concerns regarding the potential for skin irritation following repeated dermal exposure to the substance);
- Further reproductive studies, assessing general reproductive performance in rats and pre- and post-natal development in rats;
- A study assessing possible oestrogenic activity.

The committee noted the sponsor's argument (in the response to the evaluation report) regarding the NOEL and safety margin calculated by the TGA evaluator for diethylamino hydroxybenzoyl hexyl benzoate. The sponsor's agent advised that the sponsor has been asked to provide further data, if available, including the second *in vitro* dermal absorption study, data addressing oestrogenic activity and a mouse micronucleus study.

A member raised concerns that use of sunscreens in Australia differs substantially from their use in Europe. As there is a much higher exposure to sunscreens in Australia than in Mediterranean countries, for example, as consumers are encouraged to apply sunscreens every day, the potential for adverse effects following dermal absorption of sunscreens is higher in Australia.

The committee recommended rejection of the application by BASF Australia Pty Ltd for approval of diethylamino hydroxybenzoyl hexyl benzoate for use as a UVA filter in sunscreen products and cosmetics, pending consideration of further data from the sponsor addressing the concerns that were raised in the evaluation report. An evaluation of these additional data should be returned to the MEC for further consideration.

Attachment 2

Extract of MEC Minutes 6 April 2006

4.1 Diethylamino hydroxy-benzoyl hexyl benzoate (Uvinul A Plus)

The MEC, at its meeting on 6 April 2006, considered an application by BASF Australia Pty Ltd for approval of diethylamino hydroxy-benzoyl hexyl benzoate (Uvinul A Plus) for use as a UVA filter in sunscreen products. At that meeting, the MEC had recommended that the sponsor provide additional information in support of this application:

- *In vivo* oral and dermal absorption studies;
- An *in vivo* genotoxicity (mouse micronucleus) study;
- Additional dermal absorption data;
- A repeated insult patch test in humans;
- Further reproductive studies; and
- A study assessing possible oestrogenic activity.

The sponsor had provided further information in response to the MEC's requests. Members noted the TGA's evaluation of these data, and the sponsor's response to the evaluation report.

Dermal absorption

The MEC had requested that the sponsor provide an *in vivo* dermal absorption study, due to concerns relating to the previously submitted *in vitro* data. The MEC also requested a second *in vitro* study which had not been submitted before. The sponsor had submitted only the second *in vitro* dermal absorption study in pig skin, which reported dermal absorption of a 10% diethylamino hydroxy-benzoyl hexyl benzoate emulsion of 0.042%. Approximately 1% of the dose was absorbed in the previously submitted *in vitro* pig skin study (although the sponsor had argued that the absorption was only 0.1%). The evaluator had queried the results of the previous study, as data from leaky membranes were excluded from the analysis, individual values were highly variable and there was a low recovery rate.

The evaluator had concluded that the *in vitro* dermal absorption data provided by the sponsor were not adequate, as there was high variability between the two studies, and had therefore recommended that an *in vivo* dermal absorption study should be provided. Alternatively, an *in vitro* study conducted in human skin would be acceptable.

Oral absorption

The MEC had also requested an *in vivo* oral absorption study to estimate the extent of oral bioavailability in the oral toxicity studies and to estimate a safety factor. The sponsor did not provide any *in vivo* oral absorption data, and had argued that there were no systemic effects following acute or subchronic administrations of diethylamino hydroxy-benzoyl hexyl benzoate. The evaluator did not accept the sponsor's argument, as some systemic effects had been observed in oral toxicity studies – a developmental toxicity study in rats showed maternotoxicity and embryofetal effects (such as skeletal variations), and a 13-week repeat-dose oral toxicity study in rats showed liver changes attributed to metabolic adaptation. The evaluator had therefore recommended the provision of an *in vivo* oral absorption study.

- Skin irritation and sensitisation

The MEC had requested a repeated insult patch study in humans, to address the potential for skin irritation and sensitisation with repeated administration of diethylamino hydroxy-benzoyl hexyl benzoate. The sponsor had provided additional evidence to show that the substance was not a skin irritant or sensitiser on repeated administration of 10% or 20% concentrations in guinea pigs, and previously submitted data had indicated that it was not a skin sensitiser at a concentration of 25% in guinea pigs. However, the Scientific Committee on Consumer Products (SCCP) in Europe had commented that *"several questions may be raised concerning the study"* and *"the study cannot be evaluated"*. There was no skin irritation in two studies conducted in humans with concentrations of 10% or 100% of the substance, but both these studies were conducted using single patches and, hence, do not address the potential of skin irritation on repeated administration or skin sensitisation in humans. The evaluator had recommended that a repeated insult patch test in humans should be provided.

- Phototoxicity and photoallergy

Although a study in guinea pigs had shown no evidence of photosensitisation with 10% diethylamino hydroxy-benzoyl hexyl benzoate, the evaluator had noted that the number of animals used in this study was low. The sponsor had justified the number of animals on the basis that the test conditions were stringent, as UV radiation is similar to use of adjuvants in the maximisation test. The evaluator did not accept the sponsor's argument, as UV radiation is known to suppress the immune system and this would reduce reactivity of the animal, and had recommended that the sponsor provide a photosensitisation study conducted with adequate numbers of animals.

- Photomutagenicity

In response to previous concerns that metabolic activation was not used in photogenotoxicity studies, the sponsor provided a report of the International Workshop on Genotoxicity Test Procedures Working Group, which included reasons for not using metabolic activation in these assays. Following this, the evaluator had accepted that the submitted photomutagenicity assay was valid.

- Genotoxicity

An *in vivo* mouse micronucleus study showed that diethylamino hydroxy-benzoyl hexyl benzoate at two intraperitoneal doses of ~2000 mg/kg/injection did not induce an increase in micronuclei above control levels, indicating it was not genotoxic (clastogenic) in this assay. The evaluator had noted that this *in vivo* result added to the negative results seen in the *in vitro* assays assessing different end points confirming a low likelihood of genotoxic activity.

The evaluator had noted that the sponsor is conducting a mouse lymphoma assay in response to a request by the (European) SCCP for an additional genotoxicity study, and the results of that study are likely to be available early next year (2007). The sponsor should submit this study to the TGA for evaluation.

- Endocrine function

The sponsor had submitted a Hershberger assay to assess androgenic activity, which indicated that diethylamino hydroxy-benzoyl hexyl benzoate had a weak antiandrogenic effect. A uterotrophic assay conducted in rats to address the oestrogenic activity of diethylamino hydroxy-benzoyl hexyl benzoate showed that it did not show any oestrogenic effect at 250 or 1000 mg/kg/day po, but body weights were significantly lower at 1000 mg/kg/day. However, the study did not address the anti-oestrogenic activity of the substance.

 Reproductive toxicity

The MEC had requested additional studies on reproductive toxicity, as the sponsor had only submitted a developmental toxicity study in rats. The evaluator had noted that the submitted uterotrophic and Hershberger assays did not adequately address the potential for the effects of diethylamino hydroxy-benzoyl hexyl benzoate on fertility and general reproductive performance, or pre- and post-natal toxicity. The evaluator had noted that the sponsor has conducted a two-generation reproductive toxicity study in rats which might address these issues, and had recommended that these data should be submitted to the TGA.

Discussion

The evaluator had concluded that the sponsor has partially addressed the issues raised by the MEC, but had recommended that some additional studies are required. Members noted that the EU approved the use of diethylamino hydroxy-benzoyl hexyl benzoate in sunscreens in January 2005. However, there are no consistent international standards for sunscreen agents, and the EU and the FDA (US) requirements were noted to be very different, as sunscreen active ingredients are treated as cosmetics in Europe, whereas the FDA has much more extensive requirements. TGA requirements were noted to fall somewhere between those of the EU and FDA.

The committee endorsed the evaluator's conclusion that the sponsor had only partially addressed the issues originally raised by the MEC and supported the evaluator's recommendation that the sponsor should provide the following additional data in support of approval of the use of diethylamino hydroxy-benzoyl hexyl benzoate in sunscreens:

1. Provision of an *in vivo* dermal absorption study in rats (or alternatively an *in vitro* dermal absorption study conducted with human skin).
2. Provision of an *in vivo* oral absorption study in rats to estimate a safety factor.
3. Submission of the two-generation reproductive toxicity study in rats (referred to by the sponsor in the response to the evaluation report), and a mammalian cell mutation assay.
4. Provision of a repeated insult patch test in humans to directly address the potential of skin irritation and sensitisation in humans.
5. Provision of a photosensitisation study in guinea pigs conducted with an adequate number of guinea pigs.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers, the predecessor of the current SCCP.

Salivation at 200 mg/kg/day was not considered as a toxic effect by the sponsor's study report.

For safety assessment, the use of NOEL (no observed effect level: level showing no treatment-related effect at all) is preferred. The NOAEL is the no observed adverse effect level (some changes could occur at this level, but the changes are not considered biologically significant).

The SCCP used the following parameters:

Total amount of cosmetic used = 36.0 g/day; Concentration of Uvinul A Plus: 10%; total exposure of Uvinul A Plus: 3600 mg/day; dermal absorption: 0.15%; body weight: 60 kg; systemic exposure dosage = 3600 x 0.015 ÷ 60 = 0.09 mg/kg/day and NOAEL: 200 mg/kg/day.

Dermatomised; ~500 µm thickness; abdominal skin samples from 3 female donors, experiment conducted in triplicate or quadruple.

Exposed area was washed and the formulation left on the skin surface after the washing was collected with cotton swabs.

Dermatomised, ~500 µm thick skin samples from 7 female rats; experiment conducted in duplicate or single.

Exposed area was washed and the formulation left on the skin surface after washing was collected with cotton swabs.

Six membranes showing total recovery outside the range of 100 ± 15% (of the applied dose) were not considered acceptable. The total recovery in these membranes were 80%, 145%, 166%, 143%, 137% or 174%.

The study was conducted in 101 subjects (13 males and 88 females; all completed the study. The test substance was applied semi-occlusively (3/week; 48 or 72 h exposure; 0.25 mL) to the same area of the back for 24 hours. The application area was evaluated prior to each induction patch (24 or 48 hours after patch removal). After the ninth patch, the subjects were given a rest period of 10-14 days. A challenge patch (identical to the induction patch) was applied to a naive site on the back. The challenge patch was removed after 24 hours and the patch sites were observed for skin reactions at 0 and 24 hours after patch removal.

Composition (%): Uvinul A Plus 10.0; dibutyl adipate 8.0; cocoglycerides 12.0; sodium cetearyl sulfate 1.0; lauryl glucoside, polyglceryl-2 dipolyhydroxystearate, glycerin 4.0; cetearyl alcohol 2.0; glycerin 3.0; disodium EDTA 0.05; xanthan gum 0.3; magnesium aluminium silicate 1.5; phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben, isobutylparaben, aqua 1.0; and Aqua dem. ad 100.

The irradiation was in two stages, the first irradiation with UVB and then with UVA. The non-irradiated part of the back and flanks were protected from the UV rays with a cardboard mask.

Scores: 0.5 for questionable erythema (infra-erythematogenic dose); 1 for discrete or patchy erythema; 2 for moderate and confluent erythema; 3 for intense erythema.

Oestrous cycle was monitored in F0 and F1 parental females over a three week period prior to mating and continuing until evidence of mating occurred. In addition, the oestrous stage of each female (F0 and F1) was determined on the day of scheduled sacrifice.

One HD female had a squamous cyst in the uterus and did not become pregnant.

Forward mutation to TK-/- was tested using trifluorothymidine which requires TK+/- for cytotoxicity.

The concentrations were chosen based on preliminary tests at up to 4000 µg/mL. Cytotoxicity was seen at ≥31.3 µg/mL in the absence (4 h treatment) and at ≥62.5 µg/mL in the presence of S9 (4 h). Following 24 h treatment, cytotoxicity was seen at ≥7.5 µg/mL. Turbidity was seen at ≥30 µg/mL while precipitation occurred at 2000 and 4000 µg/mL.

OTC MEDICINES SECTION – TGA

SAFETY EVALUATION OF NEW SUNSCREEN ACTIVE

Diethylamino hydroxybenzoyl hexyl benzoate

Sponsor:	BASF Australia Ltd
Consultant:	s47F & Associates Pty Ltd
Proposed AAN:	Diethylamino hydroxybenzoyl hexyl benzoate
INCI name:	Diethylamino hydroxybenzoyl hexyl benzoate
Chemical name:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Trade name:	Uvinul A Plus
Lab. Codes:	99/408-1
Empirical formula:	$C_{24}H_{31}NO_4$
CAS No:	302776-68-7
Molecular weight:	397.52
Appearance:	White powder
Melting point and /or boiling point:	Melting point: 54^{0}C; does not boil at normal pressure.
Decomposition temperature:	314 ^{0}C
Solubility:	Very low in water, <0.01 mg/L after 2 hours at 20 ^{0}C and pH ~6-7.
UV spectrum:	Absorbance maximum at 354 nm
Proposed use:	UVA filter in listed suncare products at a concentration of up to 10%.

Structure:

SUMMARY OF TOXICOLOGICAL FINDINGS

In vitro percutaneous absorption assay with pig skin	up to 1%?
Absorption data for single dermal dose in rats (*in vivo*)	no data
Absorption data for single oral dose in rats (*in vivo*)	no data
Acute oral toxicity in rats	>2000 mg/kg
Acute dermal toxicity in rats	no data
Primary eye irritation in rabbits	slight irritation
Primary skin irritation in rabbits	non-irritant
Skin sensitisation (maximisation test) in guinea pigs	non-sensitiser
Photo-toxicity and photo-allergenicity in guinea pigs	negative?
Human RIPT for contact sensitisation	no data
Photosensitisation assay in humans	no data
13 weeks oral toxicity study in rats	NOEL 1350 mg/kg/day
Developmental toxicity study in rats (oral dosing)	NOEL 40 mg/kg/day
Genotoxicity Ames assay *in vitro*	negative
V79 Chinese hamster lung cell chromosomal aberration assay *in vitro*	negative
Photomutagenicity assay (Ames) *in* vitro (no metabolic activation)	negative
Photoclastogenicity assay in Chinese hamster cells *in vitro* (no metabolic activation)	negative
Cytotoxicity in BALB/C3T3 cells	negative (≤25 µg/mL)
Carcinogenicity potential	no data

Introduction

Diethylamino hydroxybenzoyl hexyl benzoate is a new UVA filter (absorbance max. ~354 nm) for use in listed sunscreen products. It is proposed that it could be used in sunscreen products (also in cosmetics) to protect against damage caused by excessive exposure to sunlight. The sponsor has stated that there are only a very few UVA absorbers available and that a commonly used UVA absorber butyl methoxydibenzoylmethane is photounstable.

According to the sponsor, diethylamino hydroxybenzoyl hexyl benzoate is an oil soluble UVA filter that can be readily incorporated in the oily phase of emulsions, and is particularly suitable for water resistant formulations. The filter is also photostable. The sponsor intends to use diethylamino hydroxybenzoyl hexyl benzoate at up to 10% in sunscreen products alone or in combination with other UV filters.

International status

Diethylamino hydroxybenzoyl hexyl benzoate was recently approved in the European Union for use in sunscreen products at concentrations of up to 10%. The chemical was included on the European Cosmetic Directive (76/768/EEC) Annex VII Part 1 in January 2005, which is the list of permitted UV filters. Approval was based on an assessment of a safety dossier by the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) of the European Commission.

According to the sponsor, the substance is "yet to be approved" for use in New Zealand, Canada, USA, UK or Sweden.

International regulatory comment

The SCCNFP is of the opinion that the use of diethylamino hydroxybenzoyl hexyl benzoate at up to 10% in sunscreen products, alone or in combination with other UV absorbers, is safe. They concluded that "diethylamino hydroxybenzoyl hexyl benzoate has low acute oral toxicity; more than 2000 mg/kg bw in the rat. A NOEL, derived from an oral 90-day study in rats is about 1350 mg/kg bw and can be applied to a safety evaluation. In a pre-natal development toxicity study, maternal toxicity was between 200-1000 mg/kg bw,
obviously due to the kind of administration (gavage as bolus in oil), while >1000 mg/kg bw can be regarded as NOEL for pre-natal development. It is not irritating to the skin and
mucous membranes in rabbits. It is not a dermal sensitiser. The percutaneous absorption was set at 0.1 µg/cm². Diethylamino hydroxybenzoyl hexyl benzoate is neither phototoxic nor photosensitising. It is not mutagenic/photo-mutagenic *in vitro*".

The SCCNFP calculated a safety margin based on a maximum absorption through the skin of 0.1 µg/cm^2 and a skin area surface (whole body) of 18000 cm^2, leading to a figure of 1.8 mg of dermal absorption of the substance per treatment ie. 0.03 mg/kg of the substance for a person weighing 60 kg. They then took the NOAEL (200 mg/kg) from the rat developmental study and divided it by the amount likely to be absorbed through the skin (exposure) of 0.03 mg/kg and came up with a margin of safety of 6667.

Interaction with other UV filters / stability

There were no data on the possible interaction of diethylamino hydroxybenzoyl hexyl benzoate with other UV filters likely to be used to formulate sunscreen products. Information on the stability of diethylamino hydroxybenzoyl hexyl benzoate showed no variation in the substance (purity: ≥99.6% in all instances; no significant changes in appearance, E value, colour, assay, odour, IR spectrum, melting point or UVA maximum spectrum) under conditions of changing storage tests at different temperature and humidity over periods of 3 months (50 ^{0}C/75% RH), 6 months (40 ^{0}C/75% RH) and 24 months (25 ^{0}C/60% RH). The acid value was decreased. Additional information on photo-stability showed thin film layers of a formulation (5% filter, 66.5% ethanol and 28.5% isopropylmyristate) irradiated with a UV dose equivalent to 6 MED (minimal erythemal dose) delivered for up to 6 hours (slides kept at 40 ^{0}C by water-cooling) did not degrade significantly: after 2-6 hours of irradiation of diethylamino hydroxybenzoyl hexyl benzoate, only 1% of the degraded substance was detected. The degraded substance was identified as 2-(monoethylamino-2-hydroxybenzoyl)-benzoic acid hexylester (loss of one of the ethyl groups of diethylamino hydroxybenzoyl hexyl benzoate).

Adverse effects

The sponsor has stated that since the substance is yet to be marketed no adverse events have been reported. According to the sponsor, no adverse effects are known and (based on the studies presented) no adverse effects are suspected.

SUMMARY/ASSESSMENT

The data package contained studies that were conducted under conditions of good laboratory practice (included quality assurance statements). Studies submitted included dermal absorption through skin from pigs (*in vitro* percutaneous), local tolerance (skin and eye irritation) in rabbits, sensitisation and photo-sensitisation in guinea pigs, acute oral toxicity in rats, repeat-dose oral toxicity in rats (13 weeks), reproductive toxicity (developmental study in rats) and *in vitro* assays on genotoxicity, photo-genotoxicity and cytotoxicity. There were no clinical studies or data on *in vivo* genotoxicity endpoints or on interaction with oestrogen receptor.

Pharmaco/toxico-kinetics

The sponsor has provided an *in vitro* study on percutaneous absorption of diethylamino hydroxybenzoyl hexyl benzoate in pig epidermal membranes. The study reported an overall percutaneous absorption of ~1% of the test substance over a period of 24 hours. However, the study had a few technical problems: some of the membranes in the study were found to be leaky and total recovery of the applied substance in some membranes was below satisfactory levels. Although the study excluded these membranes for the data analysis, variability of the individual data of the other membranes was quite high. Based on these, reliability of the study results is questionable. Please note that the SCCNFP did not consider this study valid for reasons including the leaky membrane and low recovery of the substance. The SCCNFP commented that tape stripping of the epidermis has not been done (to monitor distribution of the test substance in stratum corneum and other sublayers of epidermis).

The sponsor has conducted a second *in vitro* dermal absorption study but the study has not been submitted to the OTC Medicines Section although the study has been submitted to the SCCNFP. The SCCNFP considered the study as valid. According to the evaluation of the SCCNFP, a formulation (o/w emulsion) containing 10% diethylamino hydroxybenzoyl hexyl benzoate was tested at 2 mg/cm^2 for 24 hours on full-thickness pig skin (dermatomed skin; 500 µm thickness). According to the summary provided by the sponsor, skin layers were separated at the end of the experiment and quantity of the substance in different layers was determined (no details). In this test, percutaneous absorption was determined to be 0.1 µg/cm^2 or 0.042%. The recovery of the substance was found to be satisfactory (92.7%).

The results of the two *in vitro* studies revealed high variability not only in the individual values in one of these studies but also between studies. Thus the first study predicted a dermal absorption of ~1% whereas the second study gave a value of only 0.04%. Hence, it appears that the *in vitro* studies used in the study laboratory can give varying results on dermal absorption and hence may not be reliable. Because of the variability in the two *in vitro* studies, an *in vivo* dermal absorption study is preferable.

There was no study on the absorption of diethylamino hydroxybenzoyl hexyl benzoate via the oral route. Oral absorption study is considered necessary for a thorough assessment of exposure associated with oral repeat dose and reproductive toxicity studies in rats.

The sponsor has not provided any data for the metabolism of diethylamino hydroxybenzoyl hexyl benzoate. A document from BASF Aktiengesellschaft (dated 5 August 2004) stated that specific studies on absorption, distribution, metabolism and elimination with radiolabelled material have not been performed for the following reasons:

- In an acute oral toxicity in rats, there was no evidence of toxicity at 2000 mg/kg. In a 90-day dietary study in rats, no treatment-related effects were seen at up to 15000 ppm (1248 and 1452 mg/kg/day in males and females, respectively). Lack of toxicity in these studies could be either due to low toxicity of the substance or "lack of resorptions" (presumably lack of absorption). However, the chemical structure implies that at least a limited bioavailability can be expected after oral exposure.

- Considering the chemical structure of diethylamino hydroxybenzoyl hexyl benzoate, the metabolic pathway of the compound is predicted to be:
  - hydrolysis of the ester binding by esterases and formation of 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoic acid and 1-hexanol;
  - conjugation of the hydroxy group;
  - hydroxylation of the ethyl groups and subsequent conjugation.

- There is no specific alert that known mutagenic or carcinogenic compounds may occur by metabolic reactions. The clearly negative results of various mutagenicity and photomutagenicity studies confirm this assumption that no genotoxic metabolites are formed in the body.

- After topical application, only a negligible fraction of diethylamino hydroxybenzoyl hexyl benzoate is absorbed. The potential systemic consumer exposure is expected to be very low and of no toxicological importance.

The BASF document stated that the already available toxicity data are adequate for evaluation of the basic toxicokinetic properties of diethylamino hydroxybenzoyl hexyl benzoate including dermal penetration. According to the document, due to the favourable toxicological profile of the substance and the low dermal bioavailability, specific toxicokinetic studies with radiolabelled material are not regarded as necessary for evaluating the safety of the substance as an ingredient in sunscreen products.

The above arguments in the BASF document are not acceptable because dermal absorption cannot be estimated from the available data since there were technical problems and high variability of the data in the *in vitro* study submitted, and there was no *in vivo* study on dermal absorption. The substance is not devoid of oral toxicity. In a developmental toxicity study, toxic effects were seen at the mid and high doses (200 and 1000 mg/kg/day, respectively, orally; the effects were skeletal variations in the fetuses and maternotoxicity; see below) suggesting that the substance is absorbed orally and can cause toxic effects. Systemic toxicity may also occur following dermal exposure.

One of the predicted hydrolysis products of the substance, 1-hexanol, is reported to be of low toxicity by a US EPA report. The EPA has reclassified the list of inert ingredients in pesticide products (List 3) and included 1-hexanol (previously in List 3) in the list of "inerts of minimal

concern" (List 4). Toxicity of the second hydrolysis product, 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoic acid or of the hydroxylated metabolites (with or without conjugation) is not known.

Calculation of safety margin of diethylamino hydroxybenzoyl hexyl benzoate by the SCCNFP is present in the preceding International Regulatory comment section. The SCCNFP came up with a figure of 6667 based on a maximum absorption through the skin of 0.1 µg/cm^2 (0.042% of the applied dose), a skin area surface (whole body) of 18000 cm^2, body weight of 60 kg and a NOAEL of 200 mg/kg in a rat developmental study. However, the OTCMS evaluator's view is that the safety margin of diethylamino hydroxybenzoyl hexyl benzoate cannot be calculated at present since there is no reliable data to estimate *in vivo* dermal absorption. Even if it is assumed that the dermal absorption is ~1% based on the *in vitro* dermal study (assessed to be unreliable) submitted to OTCMS, the safety margin is only 111. In this calculation, it is assumed that oral absorption is 100% and dermal absorption is 1% (ie. dermal absorption relative to oral absorption is 1%). The estimated safety factor, calculated using a NOEL of an oral developmental study, will be lower if oral absorption is lower than 100% since the relative dermal absorption (relative to oral absorption) will be >1% and consequently the safety margin will be lower. In children, the safety margin is expected to be lower than that estimated for adults because of the higher body surface/kg body weight.

A 60 fold difference (6667 vs 111) has been noted in the estimated values of safety margins for diethylamino hydroxybenzoyl hexyl benzoate by the SCCNFP and the OTCMS evaluator. The difference is due to the differences in the values (0.042% vs 1%) used for *in vitro* percutaneous absorption, the no effect level (200 mg/kg/day vs 40 mg/kg/day), body weight (60 kg vs 50 kg) and the choice of using skin surface area or the amount of substance applied on the skin (application area of 18000 cm^2 skin vs application of 18000 mg product containing 10% excipient).

Interaction with other UV filters

There did not appear to be any information of the possible interaction of diethylamino hydroxybenzoyl hexyl benzoate with other UV filters likely to be used to formulate sunscreen products. Information on the stability of diethylamino hydroxybenzoyl hexyl benzoate was presented, but this was in isolation from other UV filters. Diethylamino hydroxybenzoyl hexyl benzoate was shown to be resistant to degradation under extremes of pH and temperature over periods of up to 3 months, and was not photodegraded significantly at up to 6 hours of UV irradiation.

Local tolerance

Based on EEC criteria, diethylamino hydroxybenzoyl hexyl benzoate was classified as a non-irritating agent to the rabbit eye, with effects (erythema at 1 and 24 hours & conjunctival discharge at 1 hour) recovering at 48 hours after application (the test material was washed out of the eye 24 hours after instillation into the conjunctivae). However, since moderate erythema was seen for up to 24 hours in 2 animals (out of 3), the substance was classified as a slight eye irritant by the OTCMS evaluator.

In a skin irritation study in rabbits, very slight erythema was observed after removal of a semi-occlusive dressing 4 hours after application of the test material. Based on a mean score of 0.1 for erythema (no oedema), diethylamino hydroxybenzoyl hexyl benzoate was determined not to be a primary skin irritant after a single application in rabbits.

In guinea pigs, there were no skin reactions in a photosensitisation test after induction with topical application of 10% or 20% of diethylamino hydroxybenzoyl hexyl benzoate but skin reactions were seen in a maximisation test for both the vehicle and test substance (25%). The reason for the skin reactions occurring even in the vehicle group (moderate or confluent erythema with oedema and incrustation) was not explained. Since skin reactions were similar for the vehicle and test substance, it appears that diethylamino hydroxybenzoyl hexyl benzoate may not be a skin irritant after a single application, at least at up to 25%.

In sensitisation and photosensitisation studies in guinea pigs, diethylamino hydroxybenzoyl hexyl benzoate did not show any sensitisation or photosensitisation response, but in the photosensitisation study the number of animals used (5 or 10 animals/group) as well as exposure (open application with massage; contact time for only 1 hour, the material applied was wiped off with a moistened cotton pad after 1 hour) was low and hence validity of the study is questionable.

In a maximisation assay in guinea pigs to assess the potential of diethylamino hydroxybenzoyl hexyl benzoate to cause contact skin sensitisation activity, appropriate numbers of animals and procedure were used, with diethylamino hydroxybenzoyl hexyl benzoate tested at a concentration of 25% during induction (in addition to 5% substance intradermally) and at challenge. Comparison with relevant control indicated that diethylamino hydroxybenzoyl hexyl benzoate did not induce skin hypersensitivity in guinea pigs at a concentration of 25%.

A repeat-dose dermal irritation study seems to have been conducted in guinea pigs but the study has not been submitted to the OTCMS for evaluation. The study has not been evaluated by the SCCNFP either. According to the Product Safety document submitted by the sponsor, diethylamino hydroxybenzoyl hexyl benzoate (Uvinul A Plus) was tested in a GLP study with guinea pigs for its potential to induce skin irritation after 14 open applications over a study period of 2 weeks. Two groups of 3 male and 3 female animals were used and the test substance (10% or 20% in propylene glycol; 50 µL to the intact skin to the right flank) or the vehicle (to the left flank) was applied daily for two weeks. Each animal was treated with one concentration of the test substance and the vehicle. Skin reactions were monitored at 24 hours after each application. The results of the study are not clear because of some confusing statements. Applications of the "20% test substance preparation respectively of the vehicle" were reported not to cause any skin reactions at up to day 7 but were reported to cause very slight or well defined erythema (grade 1 or 2; please note that open applications of the substance were used) from day 8 in "several animals" at the application sites. Skin oedema was observed in one animal at day 9 or 14, each, at the test substance treated site (revealing that the test substance was an irritant). The "10% test substance preparation respectively the vehicle propylene glycol" was reported to cause very slight erythema "in a few animals" at the application sites up to day 8. Very slight or well-defined erythema was observed for this concentration in "several animals" at

the application sites until the end of the study. The report stated that "there was no statistical difference in erythema formation between the test substance treated and the vehicle treated application sites" and therefore concluded that "the test substance did not cause skin reactions different or more severe than the vehicle". However, given the small number of animals used in the study (6 animals for each concentration), statistical significance is unlikely if only a couple of animals showed skin irritation although such irritation could be biologically relevant.

Overall, it appears that repeated application of diethylamino hydroxybenzoyl hexyl benzoate is likely to cause slight skin irritation even at a concentration of 10%, the intended concentration for clinical use.

There were no local tolerance studies in human subjects. Because of the varying effects seen in the rabbit and guinea pig studies, and the possibility of skin irritation on repeated administration (as seen in guinea pigs), a repeated insult patch test in humans is highly desirable to clarify skin irritation potential on repeated administration.

The undiluted diethylamino hydroxybenzoyl hexyl benzoate is likely to be an eye irritant but at the intended concentration of 10% in topical products, eye irritation may not occur. The animal group size (n=5 or 10/group) of the photo-sensitisation assay in guinea pigs was not large, but an absence of positive reaction in the two guinea pig studies (maximisation and photosensitisation) is reassuring.

Acute/repeat dose toxicity

An acute oral toxicity study in rats showed that diethylamino hydroxybenzoyl hexyl benzoate was of low acute toxicity (>2 g/kg). In this study, there was no evidence of toxicity with no clinical signs of toxicity, no departure from normal body weight development and no unusual lesions at necropsy. The number of animals used in the study (limit test; 3/sex) was lower than the number conventionally used (5/sex; OECD guidelines) but for an acute oral toxicity study, the low number is not considered a major deficiency.

A 13-week repeat dose oral toxicity study in rats used doses of up to 1452 mg/kg/day delivered in the diet. One HD animal had renal lesions and was killed prior to the scheduled sacrifice. The cause of death was attributed to spontaneously occurring renal lesions. However, some non-renal changes were also seen in this animal in the reproductive system (altered spermatogenesis, with degeneration or necrosis of germ cells and focal tubular atrophy in testes; atrophy of seminal vesicle and coagulation glands; hypoplasia of prostate) and heart (focal necrosis). It is likely that these changes, which occurred in only one animal (out of 20), may not be treatment-related. The NOEL was determined to be the HD (1350 mg/kg/day which is the mean of 1249 and 1452 mg/kg/day in males and females, respectively).

An observation during organ weight analysis was minor differences in relative liver weight and slightly elevated bilirubin in blood (accompanied by increased bilirubin in urine) in the HD group. Histopathological analysis did not reveal any adverse changes in the liver at any dose level compared to the control group. The change in liver weight appears to be of no toxicological concern since it was not accompanied by structural changes. It was proposed that

the increase in liver weight possibly represented an increased demand for liver function to eliminate the test substance, which appears to be a reasonable conclusion. Similarly, the decreased bilirubin levels in the blood appear to be linked to the adaptive mechanism of the liver leading to increased elimination of bilirubin.

Acknowledging a potential stimulation of liver metabolic function as a reaction triggered by diethylamino hydroxybenzoyl hexyl benzoate would suggest that oral dosing leads to some degree of systemic exposure. This possibility is further supported by changes in plasma bilirubin that are consistent with metabolism of xenobiotics and a sign of metabolic adaptation following systemic exposure to the test material. In addition, toxic effects were seen in a developmental toxicity study in rats (see below). However, the extent of oral bioavailability in the rat studies could not be estimated because kinetic data were not provided.

Reproductive toxicity

The sponsor submitted a developmental toxicity study (segment II reproductive bioassay) to address potential reproductive aspects of the dermal use of diethylamino hydroxybenzoyl hexyl benzoate in humans. In this study, mated female rats were exposed to diethylamino hydroxybenzoyl hexyl benzoate at oral doses up to 1000 mg/kg/day during the period of organogenesis (gestation days 6 through 19). Fewer mated females were pregnant in the treated groups compared to the control, but this was not due to an effect of treatment since exposure to the test material did not occur until gestation day 6. At the MD and HD (200 and 1000 mg/kg/day, respectively), maternotoxicity was seen. The effects included transient salivation and reduction of food intake at the MD and HD, and decreased body weight gain at the HD. At these two doses, higher incidences of skeletal variations were seen in the fetuses: wavy ribs at the MD and HD; notched cartilage between basisphenoid and basioccipital bones and incomplete ossification of lumbar arch in the HD group. Based on the effects seen in the maternal animals and in the fetuses, the NOEL for this study was determined to be the LD ie. 40 mg/kg/day. The NOEL was in contrast to the study report which determined NOAELs of 200 mg/kg/day for maternal toxicity (see evaluation of the study) and 1000 mg/kg/day for developmental toxicity. The study did not consider skeletal variations in the fetuses as toxic effects.

Assessment of the reproductive toxicity of diethylamino hydroxybenzoyl hexyl benzoate is very limited with only a segment II developmental study (in rat) submitted for evaluation. There was no assessment of possible oestrogenic activity.

Genotoxicity

Four *in vitro* genotoxicity assays were submitted in support of this application. Testing included two reverse mutation assays (Ames test) and two chromosomal aberration assays in Chinese hamster V79 cells. The studies were conducted with or without UV irradiation. All the assays (including the two with UV exposure) produced negative results indicating that diethylamino hydroxybenzoyl hexyl benzoate was not genotoxic *in vitro* in the presence or absence of UV radiation.

There was no *in vivo* genotoxicity test. *In vivo* tests have the advantage of taking into account absorption, distribution and excretion, which are not factors in *in vitro* tests, but are relevant to human use. In addition, metabolism is likely to be more relevant *in vivo* compared to the systems normally used *in vitro*. There are a few validated *in vivo* models accepted for assessment of genotoxicity. These include the bone marrow or peripheral blood cytogenetic assays. If a compound has been tested *in vitro* with negative results, it is usually sufficient to carry out a single *in vivo* cytogenetic assay (mouse micronucleus assay).

The *in vitro* mutation and chromosomal assays were conducted with and without UV irradiation. The assays without UV irradiation were conducted in the presence and absence of metabolic activation but the photomutagenicity and photoclastogenicity assays were conducted without metabolic activation. Since metabolites of diethylamino hydroxybenzoyl hexyl benzoate may have photogenotoxic effects, it is apparent that the photogenotoxic potential of diethylamino hydroxybenzoyl hexyl benzoate has not been fully investigated.

It was noted that in three of the genotoxicity assays (Ames test in the absence of photoirradiation; clastogenicity assays in the presence and absence of photoirradiation), cytotoxicity and precipitation of the test substance occurred at ≥20 µg/mL or µg/plate while there was no cytotoxicity or precipitation in the photomutagenicity assay (Ames test in the presence of UV irradiation) even at the highest concentration tested ie. 5000 µg/plate. The reason for the absence of cytotoxicity or precipitation in one of the genotoxic assays is not clear.

The validity of the genotoxicity tests conducted could be questioned on the grounds that diethylamino hydroxybenzoyl hexyl benzoate (at the concentrations used) might not have been able to penetrate cells to interact with genetic material. It should be noted that cell penetration is based on several factors (including the size of the molecule and lipid solubility) and an exact understanding of whether diethylamino hydroxybenzoyl hexyl benzoate penetrates into bacterial and mammalian cells appears unavailable. The presence of cytotoxicity in some of the *in vitro* assays suggested that the substance may have the ability to penetrate the cells, but it is to be noted that cytotoxicity could occur even without a substance entering the cells ie. by effects on the outer cell wall or cell membrane (eg. by affecting cell wall synthesis or permeability of cell membrane).

Overall, the inclusion of an *in vivo* assay (mouse micronucleus) would greatly enhance the credibility of the genotoxicity data package.

Cytotoxicity

An *in vitro* cytotoxicity study in BALB/C3T3 cells revealed that diethylamino hydroxybenzoyl hexyl benzoate was cytotoxic at 50 and 100 µg/mL. These concentrations were also found to be cytotoxic in three of the four genotoxicity assays (see above). The cytotoxicity study also tested phototoxicity and the substance appeared to be phototoxic at all concentrations tested (0.78-100 µg/mL) but the study considered the test substance to be non-phototoxic stating that the values of one of vehicle controls (without UV irradiation), with which the treated cells were compared, were "low". However, when the values of the test substance were compared to the untreated control, there was no evidence of phototoxicity.

Carcinogenicity

A carcinogenicity study was not submitted in the application. The following is a response by the sponsor addressing the issue of potential carcinogenicity of diethylamino hydroxybenzoyl hexyl benzoate (the OTCMS evaluator's comments are given in italics):

1. In toxicity studies, diethylamino hydroxybenzoyl hexyl benzoate showed very low toxicity even after repeated subchronic administration of high doses. In particular, no specific organ toxicity, such as liver or kidney toxicity, could be detected at any tested dose and no tissue changes were observed which might have indicated a potential oncogenicity after long-term exposure.

The conducted toxicity studies are of short duration (13 weeks or less) and do not have the potential to detect carcinogenicity potential. Although toxicity was not seen in a 13-week study, toxic effects on the developing fetuses and on maternal animals were seen in the developmental toxicity study.

2. The mutagenicity and the photomutagenicity data clearly showed that the substance or a possible metabolite have no detectable genotoxic potential.

All the genotoxic studies were conducted in vitro. There was no in vivo clastogenicity study. In photogenotoxicity studies (in vitro), metabolic activation was not used limiting assessment of potential adverse effects to the parent compound.

3. The skin tolerability of diethylamino hydroxybenzoyl hexyl benzoate in rabbits and guinea pigs was found to be high. In an acute skin irritation study with the undiluted test substance only very slight irritation was observed shortly after application. After repeated dermal administration diethylamino hydroxybenzoyl hexyl benzoate at test concentrations of 10% and 20% did not cause skin reactions different from or discernibly more severe than those observed at the skin sites treated with the vehicle alone. In addition, no signs for photoirritancy or photoallergenicity have been observed in guinea pigs. Therefore, an increased risk for skin cancer by an irritating or photoirritating effect is very unlikely.

The repeated dermal irritation study in guinea pigs was not submitted for evaluation. It appears from the limited information given that both the 10% and 20% concentrations of the test substance showed signs of skin irritation on repeated administration. Please note that the intended concentration of diethylamino hydroxybenzoyl hexyl benzoate in topical products is 10%. The number of guinea pigs used in a photoirritancy-photoallergenicity study is low and hence validity of the study results is questionable.

4. There is no information available that diethylamino hydroxybenzoyl hexyl benzoate might adversely change the protective properties of the epidermis (such as an influence on optical properties).

The substance appears to show irritation potential in guinea pigs (on repeated application) and was cytotoxic in in vitro genotoxic assays and in a cytotoxicity assay, and hence the sponsor's statement is not supported.

5. *In vitro* penetration experiments with human skin showed that the substance when applied in a sunscreen formulation remains almost entirely in the horny layer and is not likely to reach the living parts of the epidermis or dermis.

An vitro dermal absorption study in pig skin has been submitted but the study was assessed to be inconclusive. The sponsor has not submitted to the OTCMS (or to SCCNFP) any study conducted with human skin and hence the claim that the substance remained entirely in the horny layer of the "human" skin could not be verified. A second in vitro study seems to have investigated the distribution of the substance in the layers of pig skin but the study has not been submitted. Because of variability in the results between the two studies in pig skin, an in vivo dermal absorption study is preferable.

6. Photostability data indicate that the compound is not photodegraded or releases free radicals in presence of UV light. A photomutagenic effect by free radicals or by degradation products is therefore not expected. Although no carcinogenic assay has been performed to specifically test the carcinogenic or photocarcinogenic properties, the available data clearly indicate that an increased risk of a carcinogenicity even following UV exposure is not to be expected.

The sponsor did not include metabolic activation system in an in vitro photomutagenicity or photoclastogenicity assay and hence it is not known whether metabolites formed are photogenotoxic.

7. The chemical structure of the substance does not give alert of a close relationship to known chemical or photochemical carcinogens.

The sponsor's comment appears to be reasonable.

The sponsor's report concluded that a long-term carcinogenicity and photocarcinogenicity study for diethylamino hydroxybenzoyl hexyl benzoate is considered not to be of high priority to the sponsor. The sponsor's report expected that the substance would provide protection against UV induced skin tumours in such an experiment. However, as seen above, most of the sponsor's arguments for not providing a carcinogenicity are not convincing and hence it is considered that the potential for carcinogenicity of diethylamino hydroxybenzoyl hexyl benzoate has not been adequately addressed. It is noted that diethylamino hydroxybenzoyl hexyl benzoate was not genotoxic in a limited series of *in vitro* studies including photomutagenicity assays (but without metabolic activation) but absence of an *in vivo* clastogenicity study is a concern.

It would appear that no direct data on the metabolism (ADME study) are available for diethylamino hydroxybenzoyl hexyl benzoate so the potential for the formation of active metabolites is not accurately known.

In the 13-week repeat dose toxicity study in rats, although the findings indicated no apparent histopathological evidence of neoplastic changes, the period of exposure was extremely short and unlikely to induce neoplastic changes. However, in the oral developmental study in rats, there was some evidence of adverse effects on the developing fetus (skeletal variations) following exposure during organogenesis. Maternotoxicity was also seen at the doses which caused developmental toxicity and hence it is not clear whether developmental toxicity was the direct effect of the test substance or caused indirectly by maternotoxicity. There was no assessment of interaction of diethylamino hydroxybenzoyl hexyl benzoate with oestrogen receptor.

Diethylamino hydroxybenzoyl hexyl benzoate (10% and above, topical; concentrations below 10% were not tested) was only a slight skin irritant but was not a skin sensitiser (or photosensitiser in a limited number of animals), which would indicate negligible interaction of the test material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and, potentially, to DNA.

A non-genotoxic mechanism for carcinogenesis involving chronic skin irritation and inflammation could be relevant for topically applied substances such as UV filters in sunscreens. Data/information available from skin irritation studies (rabbits and guinea pigs) indicate diethylamino hydroxybenzoyl hexyl benzoate may not be an irritant at the intended concentration of 10% after a single administration, but it could be an irritant on repeated dermal exposure.

Overall, the sponsor has not provided adequate justification for not providing a long-term carcinogenicity study.

RECOMMENDATION

The sponsor has submitted a package of data containing studies on diethylamino hydroxybenzoyl hexyl benzoate that conform to GLP and OECD standards or acceptable guidelines regarding conduct of studies (includes QA statement). Diethylamino hydroxybenzoyl hexyl benzoate is a UV-A filter that has been on the accepted list of UV filters in the EU since January 2005 at a concentration of up to 10% in products.

There was no definable toxicity detected in a 13-week repeat-dose oral toxicity study in rats at doses up to ~1350 mg/kg/day (no-effect level, NOEL). In a developmental toxicity study in rats, skeletal variations were seen in the fetuses (200 and 1000 mg/kg/day) but only in the presence of maternotoxicity (transient salivation and reduced food consumption at 200 and 1000 mg/kg/day; reduced body weight gain at 1000 mg/kg/day). The NOEL was 40 mg/kg/day. The substance was not a skin irritant but was a slight eye irritant in rabbits. In a sensitisation study in guinea pigs, the substance (25%) was not a skin sensitiser. *In vitro* genotoxicity studies examining potential adverse effects of diethylamino hydroxybenzoyl hexyl benzoate on structural genetic material were negative.

The evaluation of the studies submitted revealed the following concerns:

1. The safety margin (the NOEL of 40 mg/kg/day in the developmental toxicity study in rats vs. intended exposure of the chemical) was only 111, which is just adequate (a safety margin of 100 is the minimum requirement).
2. There is a potential for skin irritation following repeated administration of the substance. Although not a skin irritant in rabbits after a single administration, repeated dermal exposure appears to cause skin irritation as reported in a summary provided for a study in guinea pigs. The full study report for the repeated dose dermal study in guinea pigs has not been submitted for assessment.
3. The submitted *in vitro* dermal absorption study was unreliable to estimate dermal absorption of diethylamino hydroxybenzoyl hexyl benzoate. *In vivo* kinetic studies are required to estimate dermal and oral absorption of the substance. Overall, toxicokinetic data provided was inadequate.
4. There was no *in vivo* genotoxicity study in the sponsor's submission. The justification provided for the absence of carcinogenicity data was not adequate. An *in vivo* genotoxicity study would at least give greater degree of assurance that the compound does not have the potential to be a genotoxic carcinogen. An *in vivo* mouse micronucleus study has been identified as establishing a greater degree of assurance regarding assessment across appropriate genotoxic end points.
5. Assessment of reproductive toxicity was limited to developmental toxicity. The potential for oestrogenic activity was not addressed.
6. A phototoxicity/photosensitisation study in guinea pigs was considered inconclusive because of the low exposure to the substance and low number of animals used.
7. In photogenotoxicity studies, metabolic activation was not used.

Based on the above concerns, the sponsor's application is **not supported**. The advice/opinion of the committee is requested.

EVALUATION OF SUBMITTED TOXICITY DATA

TOXICOKINETIC/PHARMACOKINETIC DATA

2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester - Dermal absorption through pig epidermal membranes in vitro. BASF Aktiengesellschaft. Germany; project no. 51H0408/992300; C. Wiemann, J. Hellwig and E. Leibold; 2000; GLP/QA-yes.

The *in vitro* absorption of diethylamino hydroxybenzoyl hexyl benzoate from a sunscreen formulation (o/w emulsion containing 10% diethylamino hydroxybenzoyl hexyl benzoate) across pig epidermis (includes stratum corneum) was examined. The test formulation was applied to prepared epidermal membranes (8 or 12 samples/test substance concentration; 2/vehicle control) which were positioned across a chamber that contained a receptor fluid. The test material was applied at a nominal rate of 2 or 10 mg/cm^2 (equivalent to 200 or 1000 µg diethylamino hydroxybenzoyl hexyl benzoate/cm^2) and left in contact for a period of 24 hours. The rate and extent of absorption of the test substance were determined by removing samples of the receptor fluid at 0 (pre-test), 0.5, 1, 3, 6, 12, 20 and 24 hours after application of the test substance. At the end of the experiment, test substance remaining on the surface of the skin was collected by washing off. Skin stripping was not performed to monitor the distribution of diethylamino hydroxybenzoyl hexyl benzoate in stratum corneum or other structures of the epidermis. The concentration of test substance in the receptor fluids, the epidermis, and in the donor and receptor washings was analysed by HPLC.

Seven membranes (2 treated with the low concentration and 5 treated with the high concentration) were found to be leaky and/or showed low recovery rate (<80%). All these membranes were not used for data analysis. Data presented showed mean absorption rates of 0.01 and 0.34 µg/cm^2/h for the low (200 µg/cm^2) and high concentrations (1000 µg/cm^2) of diethylamino hydroxybenzoyl hexyl benzoate, respectively. The overall absorption (total content in the recovery fluid, and in the washings of the recovery chamber; mean $\pm$ SD) over 24 hours was 0.9 $\pm$ 0.5% (2.2 µg/cm^2) and 1.1 $\pm$ 1.2% (12.5 µg/cm^2) of the applied dose, for the low and high concentrations of the test substance, respectively. The concentrations present in the epidermal membrane (after washing of the skin surface) at the end of 24 hours were 10.5% and 6.2% of the applied dose, for the low and high concentrations, respectively.

Total recovery of the originally applied dose was 102% for the low concentration and 91% for the high concentration (excluding data for membranes which were found to be leaky or when recovery was found to be <80%), which was made up of the amount in the receptor fluid, the amount in the epidermis and the amount removed from the epidermis after the 24 hours exposure period.

Analysis of the individual data revealed high variability in the values, especially for the high concentration (see SD, above). For the low concentration, the lowest and highest individual values for cumulative absorption showed a ~20 fold variability (values: 0.0240 and 0.5469 µg/cm^2) while for the high concentration, the variability was ~500 (values: 0.0432 and 22.3335 µg/cm^2).

Based on the data, it appears that diethylamino hydroxybenzoyl hexyl benzoate has low dermal absorption. However, because of the technical problems with some membranes (leaky membrane; low recovery rate) and high variability of the data, results of this study are questionable.

ACUTE TOXICITY

> 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester - Acute oral toxicity study in Wistar rats; BASF Aktiengesellschaft. Germany; project no. 10A0408/991123; A.O. Gamer and H.D. Hoffman; 2000; GLP/QA-yes.

A single group of 3/sex Wistar rats (approx. 8-16 weeks old) was used in this acute oral toxicity study, where diethylamino hydroxybenzoyl hexyl benzoate (batch no. R323/681) was administered by gavage at 2000 mg/kg. The test material was suspended in 0.5% sodium carboxymethylcellulose in distilled water ('Tylose CB 30.000 in Aqua bidest'). The animals were fasted prior to administration of the test material. Animals were observed for a period of 14 days following the administration of the test material. Parameters checked included clinical signs of toxicity, body weight, mortality and an examination for gross lesions at necropsy.

No deaths occurred and no clinical signs of toxicity were observed during the course of the study. Body weight development was within the normal range for all animals and there were no abnormal findings at necropsy. The oral LD_{50} value was determined to be >2000 mg/kg.

The study has been reported to be conducted following the guidelines of OECD (no. 404), EC and USEP. However, the number of animals used in the study was low according to the OECD guidelines which recommend 5 animals/sex.

LOCAL TOLERANCE/PHOTOTOXICITY EFFECTS

> 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesaeurehexylester - Acute eye irritation in rabbits; BASF Aktiengesellschaft, Germany; project no. 11H0408/992236;
> C. Wiemann and J. Hellwig; 2000; GLP/QA-yes.

The primary ocular irritation potential of diethylamino hydroxybenzoyl hexyl benzoate (batch no.R 323/681) was examined following instillation of 0.1 mL into the conjunctival sac of one eye of each of 3 young adult NZ white rabbits (1 male and 2 females), while the untreated eye served as a control. The treated eyes were rinsed with tap water at 24 hours after application. Assessment (scoring) of the degree of ocular irritation took place at 1, 24, 48 and 72 hours after application of the test material. Estimation of the irritation potential involved using the scores from each reading point (excluding scores at 1 hour) and calculating the respective mean values for each type of ocular lesion. All individual scores at 24, 48 and 72 hours were totalled and then averaged.

The results showed that slight (in one animal, score = 1) or moderate erythema (in 2 animals, score = 2) of the conjunctivae was seen up to 24 hours, and conjunctival discharge was seen in

the male at 1 hour. There were no symptoms at 48 and 72 hours inspections. Iris and cornea were not affected. The mean score was 0.3 for conjunctival redness.

Based on the 93/21/EEC criteria for classifying ocular irritation, the study concluded that the test substance did not show any indication for eye irritation potential. However, based on the signs (especially moderate erythema), the test substance should be considered as a slight eye irritant.

> 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesaeurehexylester - Acute dermal irritation / corrosion in rabbits; BASF Aktiengesellschaft, Germany; project no. 18H0408/992237; C. Wiemann and J. Hellwig; 2000; GLP/QA-yes.

The primary skin irritation potential of undiluted diethylamino hydroxybenzoyl hexyl benzoate (batch no. R 323/681) was assessed in an assay using topical application of 0.5 g of the test material to an area of 6 cm^2 on clipped intact dorsal skin of 3 NZ white rabbits (2 males, 1 female). The treated site was covered with a patch and a semi-occlusive dressing. Exposure to the test material lasted for 4 hours after which time the dressing was removed and the test site was washed with polyethylene glycol and a mixture of polyethylene glycol and water (1:1). Irritation scores were determined at 1, 24, 48 and 72 hours after removal of the dressing. The individual irritation scores for each type of induced lesion for each of the assessment times were used to calculate a primary skin irritation index.

Very slight erythema (score = 1) was seen in two males at 1 hour and in one male at 24 hours. The mean score for erythema (out of a maximum score of 4) was 0.67 at 1 hour and 0.33 at 24 hours. There was no oedema. Based on the calculation of the mean of erythema and oedema according to the EEC Commission Directive 93/21/EEC (only the readings of 24, 48 and 72 hours were used; mean score for erythema = 0.1, oedema = 0), the study concluded that the test substance was non-irritating.

> 2-(4-diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester - Maximisation test in guinea pigs; BASF Aktiengesellschaft, Germany; project no. 30H0408/992238; C. Wiemann and J. Hellwig; December 2000; GLP/QA-yes.

A preliminary concentration range-finding study was conducted in female Harlan guinea pigs pretreated with FCA (4 weeks prior to the testing of the test substance) to establish the highest 'non-irritating' test article concentration to be used for the challenge in the main study. A concentration of 25% was selected for the main study based on the results with 10% and 25% concentrations. At 10% and 25%, slight to moderate skin irritation (discrete and patchy erythema or moderate and confluent erythema; not concentration-dependent) was evident immediately after removal of a 24-hour occlusive patch but there were no skin reactions at 24 hours after patch removal. The preliminary study also established 5% concentration (showed moderate irritation) as appropriate for intradermal induction. A vehicle control was not used and hence it is not clear whether the skin reactions seen after the test substance were treatment-related. Please note that 'skin reactions' were seen after topical administration of the vehicle in the main study (see below).

In the main study (Magnusson and Kligman), groups of female Harlan (Hsd Proc: DH) SPF guinea pigs were subjected to an induction and challenge phase with diethylamino hydroxybenzoyl hexyl benzoate. A treated group (n=10) and a control (n=5) group were included in this assay. Data from a non-concurrent positive control group (January 2000) was used to validate the sensitivity of the species and strain of animal used.

Induction involved administration of the test material intradermally at a concentration of 5% (vehicle: olive oil) followed by topical application of 25% test material under an occlusive dressing (see below). At least 2 hours before each test substance application an area of dorsal skin was clipped free of fur. During the induction Freund's Complete Adjuvant (FCA) was employed as a vehicle for the test material to sensitise the induction system. Three pairs of intradermal injections (0.1 mL/site) were carried out on test day 1. In the test group animals, these 3 sites coincided with application of FCA (site 1), the test material and FCA (site 2) and the test material alone (site 3). The control group received FCA plus the vehicle olive oil or just FCA (in saline). Parameters assessed during the study included formation/occurrence of erythema and/or oedema, which was graded according to the grading scale of Magnusson and Kligman. One week after the intradermal induction, a gauze patch (8 cm^2) containing the test substance (1 mL of 25% test substance in olive oil) was applied for 48 hours under occlusive dressing over the three injection sites (pre-clipped) located on the dorsal region of the test animals. Skin reactions were monitored at 24 hours after intradermal injections and immediately after removal of the topical patch.

At challenge (14 days after epicutaneous induction) the test material was applied topically (occlusive dressings) to a naive site at 25% (0.5 mL) to try and elicit a response. The vehicle was applied to another site. Application and fixing of the patches involved the same method described for fixing of the induction patches except that the gauze patch size was 4 cm^2. These challenge patches were left in place for 24 hours. Scoring of skin reactions to the challenge treatments took place 24 and 48 hours after removal of the patches and followed the Magnusson and Kligman scaling system.

The study maintained a second control group (control group 2) intended for a potential second challenge in case of borderline results at the first challenge. The group was treated with the vehicle and FCA as in the other control group (control group 1) but was challenged only with the vehicle. According to the study report, this group was not treated with the test substance since a second challenge was not considered necessary on the basis of unambiguous results of the challenge (in the treated group).

During the induction phase with intradermal administrations, moderate or confluent erythema and oedema were seen at the sites treated with the vehicle or test substance (score: 2E in all animals) suggesting that the reactions seen were due to the vehicle or the intradermal procedure. At the sites treated with intradermal FCA or test substance plus FCA, severity of the skin reactions was slightly higher (intense erythema and swelling; score: 3 in all animals).

After the topical application (induction phase, immediately after patch removal), all the animals had moderate or confluent erythema with oedema and incrustation (score: 2E+) irrespective of the treatment (vehicle or test substance). The reason for skin reactions occurring after topical

application of even the vehicle was not explained in the study report. It is not clear whether skin reactions after the intradermal injections persisted at the time of evaluation of the topical administration site.

After challenge, none of the animals in the control or treated group showed any skin reactions at 24 or 48 hours after patch removal (skin reactions were not monitored immediately after patch removal).
All animals had body weights within the normal range for the species and strain. Necropsies were not conducted.

In a maximisation test, the non-current positive control group (induction with 5% or 10% alpha-hexylcinnamaldehyde in paraffin oil or Lutrol E 400; challenge with 5%) had generated 'positive skin reactions' at challenge (in 10/10 animals at 24 hours after patch removal, first or second challenge; in 5 or 9 animals at 48 hours; skin reactions were not stated) in this species and strain of animal. The positive control (induction with 25%; challenge with 10% or 15%) was also a sensitiser in a Buehler test.

Overall, the study showed that diethylamino hydroxybenzoyl hexyl benzoate was not a skin sensitiser at a challenge concentration of 25% applied topically.

Note: Skin reactions were seen even after topical application of the vehicle during the induction phase.

> Phototoxic and photoallergenic potential by cutaneous route in guinea pigs. Centre International de Toxicologie. Germany; Study no. 20938 TSG, X. Manciaux; 26 March 2001; GLP/QA-yes.

This study assessed the photo-allergic potential of diethylamino hydroxybenzoyl hexyl benzoate in a cutaneous sensitisation assay using four groups of male Hartley Crl: (HA) BR guinea pigs:

Group 1: Untreated control (no vehicle or test substance) + UV irradiation
Group 2: Vehicle control + UV irradiation
Group 3: Test substance (no irradiation)
Group 4: Test substance + UV irradiation

Only 5 animals/group were used except Group 4 which had 10 animals.

In the induction phase, 0.2 mL diethylamino hydroxybenzoyl hexyl benzoate (10% on the first day; 20% on other days) or vehicle (olive oil) was applied epicutaneously six times over a period of 8 days to a previously shaved area of skin, approximately 9 cm^2 in size. Each application (open application followed by a gentle massage) lasted for 1 hour, after which any residual test substance was wiped off with a moistened cotton pad. The animals (except Group 3) were irradiated approximately 30 minutes after treatment with the test substance or vehicle. The irradiation was 'infra-erythematogenic' (score of erythema ≤ 0.5), the dose being 9 Joules/cm^2 for UVA and 0.1 Joules/cm^2 for UVB. The non-irradiated parts were protected from the UV rays.

The phototoxic potential of the test substance was tested in all animals before and 1, 2 and 24 hours after the first treatment and/or irradiation. Cutaneous reactions were also monitored at 24 hours after each subsequent irradiation. After the induction phase (after the 6th application of the test substance), the animals were free of any treatment and/or irradiation for a period of 20 days (days 9-28). On day 29, challenge was given (except to Group 1: the non-treated control group) with 0.2 mL of 10% test substance (or vehicle to the vehicle control group) which was applied topically to two naive areas (4 cm^2; on the left and right flanks). After a minimum of 30 minutes after treatment, the animals (except Group 3: the non-irradiated group) were irradiated: the left flank with UVB and the right flank with UVA, at a level which did not cause erythema. Cutaneous reactions were scored before and 1, 4, 24 and 48 hours after the challenge application and/or irradiation.

In this study, the concentrations of the test substance were selected from a preliminary study in which guinea pigs were tested with 10% or 20% test substance (any residual test substance was reported to be wiped off with a moistened cotton pad, but it was not stated when the substance was wiped off and hence the duration of exposure is not clear; scoring at 1, 6 and 24 hours). Both the concentrations were not irritants (no skin reactions).

In the main study, skin reactions in the treated groups (with or without irradiation) during the induction phase or after challenge were comparable to the controls, with mean severity <1.0 in all groups (1 = weak erythema; 0.5 = questionable erythema). The study suggested that the test substance was not an irritant, phototoxic or photoallergic. However, it was noted that the number of animals used in the study was low (5 or 10/group). Non-concurrent positive controls from the study laboratory (conducted 1 or 4 years prior to the study date; 8-methoxypsoralen 0.1% in corn oil; chlorpromazine 0.05% - 0.1%) showed that the animal model was appropriate to detect photosensitisation.

Over the course of the study there were no deaths or treatment-related changes in body weight or clinical signs. Skin samples were collected but were not examined histologically.

Note: The number of animals used was low.

GENOTOXICITY

> Salmonella typhimurium/Escherichia coli reverse mutation assay (Standard plate test and preincubation test) with 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester; BASF Aktiengesellschaft, Germany; project no. 40MO408/994145; H.D. Hoffman and G. Engelhardt; June 2000; GLP/QA-yes.

The mutagenic potential of diethylamino hydroxybenzoyl hexyl benzoate (batch no. R 323/681) was examined in the Ames test using standard plate incorporation and pre-incubation methods. In the assays 4 strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 uvrA) were used to assess possible point mutation effects. The assay incorporated metabolic activation prepared from treated rat livers, and positive and vehicle control groups were included in the study design. Positive controls included 2-aminoanthracene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine and 4-

nitroquinoline-N-oxide, with results for these agents generating significantly greater mutant frequencies across all strains.

The concentrations of the test material (vehicle: DMSO) were based on the generally recommended highest test concentration for non-toxic substances, which is up to 5000 µg/plate in the presence and absence of metabolic activation. Cytotoxicity was seen at ≥500 µg/plate in the plate test and at ≥100 µg/plate in the preincubation assay. Precipitation occurred at ≥500 µg/plate.

There was no evidence of an increase in the number of revertant colonies for any of the cultured strains in the presence or absence of metabolic activation. The mutant frequencies were in the range seen in the historical controls. The positive control induced a significant increase in the frequency of mutant colonies verifying the sensitivity of the strains used. Diethylamino hydroxybenzoyl hexyl benzoate was shown not to possess mutagenic activity in this assay.

> In vitro chromosome aberration assay with 2-(4-diethylamino-2-hydroxybenzoyl)-benzoesäure-hexylester; BASF Aktiengesellschaft, Germany; project no. 32M0408/994163; G. Engelhardt and H.D. Hoffmann; November 2000; GLP/QA-yes.

Diethylamino hydroxybenzoyl hexyl benzoate (vehicle: DMSO) was evaluated for potential clastogenic effects on cultured V79 Chinese hamster lung cells in the presence and absence of metabolic activation (rat liver S9). In pre-testing (1-4000µg/mL, 4 hours exposure, 18 hours harvest time), concentrations ≥50 µg/mL were cytotoxic as seen by a decreased mitotic index and/or number of cells. Precipitation was also seen at these concentrations.

In the main test, the study attempted to use 5-500 µg/mL of the test substance and aimed to use three concentrations of the test substance for data analysis, but because of cytotoxicity (reduction in the number of analysable metaphases, cell count and mitotic index), the concentrations for evaluation could not be selected and the slides were not evaluated. Hence the first main study (4-hour exposure of the test substance; 18 hours harvest time) was repeated with 2.5, 5, 10, 20, 40, 80 and 160 µg/mL (±S9 mix) of the test substance but only 5, 10 and 20 µg/mL (in the absence of S9 mix), or 10, 20 and 40 µg/mL (in the presence of S9 mix) were evaluated for clastogenicity because higher concentrations were reported to reduce either the number of analysable cells or available metaphases ("evaluation not possible"). Similarly, in a second experiment, up to 20 µg/mL in the absence of S9 mix (18 hours exposure; 18 or 28 hours harvest time) and up to 80 µg/mL in the presence of S9 mix (4 hours exposure; 28 hours harvest time) were tested, but only some concentrations were assessed (see table below) because of cytotoxicity.

The incidence of aberrant metaphases in the cells treated with the test substance was comparable to either the vehicle control or the historical control. Appropriate positive controls (showed positive response; ethyl methanesulfonate 350 µg/mL without S9 mix and cyclophosphamide 0.5 µg/mL with S9 mix) and vehicle control were included in the study. The test substance was not clastogenic under the conditions of the study.

Chromosomal aberration assay in V79 Chinese hamster lung cells

Exposure of test substance (hours)	Harvest time (hours)	S9 mix	Test substance (µg/mL)	Mitotic index (%), relative to vehicle control	Cell count (%), relative to control	% Aberrant metaphases	
						Excluding gaps	Including gaps
First experiment							
4	18	-	Vehicle	100	100	1.5	2.0
4	18	-	5	83	82	2.5	4.0
4	18	-	10	121	95	1.5	3.0
4	18	-	20	46	85	1.5	3.0
4	18	-	Hist.control			1.8	4.1
4	18	+	Vehicle	100	100	0	2.0
4	18	+	10	143	95	2.0	3.0
4	18	+	20	143	87	2.5	4.5
4	18	+	40	141	83	2.0	4.0
4	18	+	Hist.control			2.0	5.3
Second experiment							
18	18	-	Vehicle	100	100	4.5	6.0
18	18	-	2.5	92	92	1.5	4.0
18	18	-	5	95	63	2.0	9.0
18	18	-	10	84	78	1.0	7.0
18	18	-	Hist.control			2.0	6.3
18	28	-	Vehicle	100	100	2.5	6.0
18	28	-	10	31	65	3.0	5.5
18	28	-	Hist.control			2.4	5.3
4	28	+	Vehicle	100	100	2.5	6.0
4	28	+	10	86	107	2.0	3.5
4	28	+	20	157	83	4.0	7.5
4	28	+	40	131	64	3.0	6.5
4	28	+	Hist.control			2.1	4.6

Vehicle: DMSO. Number of metaphases analysed: 200 for each treatment. Hist.control: Historical control for DMSO (mean values).

Photomutagenicity in a Salmonella typhimurium and Escherichia coli reverse mutation assay with 2-(4-diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester; RCC Cytotest Cell Research GmbH, Germany; project no. 663701; A. Sokolowski; 18 October 2000; GLP/QA-yes.

Diethylamino hydroxybenzoyl hexyl benzoate was assessed for its potential to induce photomutagenicity in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1537 and in *Escherichia coli* WP2 in the absence of metabolic activation. Both plate incorporation and pre-incubation assays were used. Concentrations of diethylamino hydroxybenzoyl hexyl benzoate tested ranged from 33 to 5000 µg/plate and UVA/UVB radiation (up to 200 mJ/cm^2 of UVA or 10 mJ/cm^2 of UVB) was used to investigate possible induction of photo-mutagenicity. The UV dose was selected as a dose inducing an approximate increase in conversion frequency of 2 fold

over background levels. Positive (8-methoxypsoralen 12.5 µg/plate; 4-nitro-o-pheylene-diamine 50 µg/plate; sodium azide 10 µg/plate) and vehicle (DMSO) controls were included in this assay. Criterion for acceptance of a substance as a photo-mutagen was a concentration-related and reproducible increase in the number of revertants (the test substance was not tested in the absence of irradiation). No toxic effects or precipitation occurred with any of the test concentrations. The data showed that there were no photo-mutagenic effects of diethylamino hydroxybenzoyl hexyl benzoate, with the frequencies of convertants similar in controls and test cultures. A clear response to the positive control verified the sensitivity of the test system.

Note: There was no metabolic activation. The reason for the absence of cytotoxicity or precipitation is not clear.

> Chromosome aberration test in vitro: photomutagenicity in Chinese hamster V79 cells with 2-(4-diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester; RCC Cytotest Cell Research GmbH, Germany; project no. 663702; A. Czich; November 2000; GLP/QA-yes.

Diethylamino hydroxybenzoyl hexyl benzoate (vehicle: DMSO) was evaluated in two experiments for potential clastogenic effects on cultured V79 Chinese hamster lung cells in the absence of metabolic activation. The cultures were pre-incubated with the test substance (up to 80 µg/mL in the first experiment and up to 40 µg/mL in the second experiment) for 30 min. After exposure to UV light (225/11.25 mJ/cm^2 UVA/UVB in experiments I and II; or 375/18.75 mJ/cm^2 UVA/UVB in experiments II) and incubation for a further 3 hours the cultures were washed twice. Corresponding cultures with the test substance were kept in the dark for 3 hours (non-irradiated). Eighteen (exposure I) or 28 hours (experiment II) after start of treatment, the cultures were prepared for cytogenetic evaluation. Two hundred metaphase plates (per test substance concentration, with or without irradiation) were scored for structural chromosome aberrations.

Precipitation and cytotoxicity were seen at ≥20 µg/mL but there was no evidence of photoclastogenicity at up to 80 µg/mL of the test substance. Appropriate positive controls (showing positive response; ethyl methanesulfonate 1200 µg/mL or 8-methoxypsoralene 0.5 µg/mL) and vehicle control were included in the study. The test substance was not clastogenic under the conditions of the study.

Note: There was no metabolic activation.

CYTOTOXICITY

> Cytotoxicity assay *in vitro* with BALB/C3T3 cells: Neutral red (NR) assay with 2-(4-diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester at simultaneous irradiation with artificial sunlight. RCC Cytotest Cell Research GmbH, Germany; project no. 683301; M. Gloss; 12 January 2001; GLP/QA-yes.

The assay was based on the uptake of a dye neutral red and its accumulation in the lysosomes of viable uninjured cells. The dye was extracted from cells after lysing with a mixture of deionised water, ethanol and glacial acetic acid, and quantified photometrically at 540 nm.

BALB/c 3T3 clone 31 cells (fibroblast cell line from mouse embryo) were initially treated with various concentrations of the test substance (8 concentrations, up to 100 µg/mL in DMSO). After 1 h pre-incubation with the test substance or the positive control (chlorpromazine; 6.25-200 µg/mL in the absence of irradiation and 0.125-4 µg/mL in the presence of irradiation), the cells were irradiated with artificial sunlight for 50 minutes with 5 J/cm^2 UVA (1.7 mW/cm^2). Parallel cultures were kept in the dark for 50 minutes.

No precipitation was seen in the incubation medium (Earle's Balanced Salt Solution). When compared to the vehicle control, cytotoxicity at 100 µg/mL in the absence of irradiation, and phototoxicity (all concentrations in the presence of UV irradiation showed lower % values when compared to the % values without irradiation; see table below) were seen. However, the study concluded that the test substance was not phototoxic stating that the solvent control in the cells treated without irradiation had lower values (hence the % values for the test substance in the absence of UV irradiation seemed to be higher).

When compared to the untreated control (no vehicle), cytotoxicity was evident at 50 and 100 µg/mL but there was no evidence of phototoxicity since the % viability was comparable in the presence and absence of UV irradiation (see values in parenthesis in the table below).

Concentration (µg/mL)	**Without UV irradiation**		**With UV irradiation**	
	$OD_{540\ nm}$	% of solvent control[#]	$OD_{540\ nm}$	% of solvent control[#]
Untreated control (no vehicle)	1.047	126	0.849	93
Vehicle control	0.833	100	0.917	100
0.78	1.022	123 (98)	0.856	93 (100)
1.56	1.017	122 (97)	0.839	91 (98)
3.13	1.004	120 (95)	0.844	92 (99)
6.25	1.052	126 (100)	0.821	89 (96)
12.5	1.001	120 (95)	0.804	88 (95)
25	0.990	119 (95)	0.768	84 (90)
50	0.895	107 (85)	0.709	77 (83)
100	0.666	80 (64)	0.450	49 (53)

[#]The figures in parentheses denote the % relative to the untreated negative control. Statistical tests were not conducted.

Based on the results, diethylamino hydroxybenzoyl hexyl benzoate is assessed to be cytotoxic at 50 and 100 µg/mL. Because of the uncertainty of the effects of the vehicle control, the potential of phototoxicity cannot be assessed from the study results.

REPEAT-DOSE/SUBCHRONIC TOXICITY

2-(4-diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester. Subchronic toxicity study in Wistar rats. Administration in the diet for 3 months. BASF Aktiengesellschaft, Germany; project no. 50S0408/99093; W. Mellert *et al.*; 14 March 2001; GLP/QA-yes.

In this study, groups of Wistar rats (10/sex/group) were administered diethylamino hydroxybenzoyl hexyl benzoate in the diet at 0, 600, 3000 or 15000 ppm for a period of 3 months. Based on food intake, the amounts of test substance were calculated to be ~52, 250 and 1249 mg/kg/day in males and ~59, 288 and 1452 mg/kg/day in females, for the 600, 3000 and 15000 ppm groups, respectively. Control animals received ground diet only. Parameters measured/assessed during the course of the study included clinical signs of toxicity, survival, ophthalmology, functional observational battery (home cage and open field observations; sensorimotor tests/reflexes), locomotor activity, body weights, food intake, haematology, clinical chemistry, urinalysis, organ weights, and micro- and macroscopic examination of tissues at scheduled or non-scheduled sacrifice. Immediately after necropsy and organ weight determination, the right testis and cauda epidermis were taken from all male animals and sperm analysis (motility, head count and morphology) was conducted. The study did not include a recovery phase (treatment free recovery period).

All animals except one survived to scheduled sacrifice with no overt evidence of systemic toxicity based on an absence of treatment-related clinical signs of toxicity. One HD male had a palpable mass in the abdomen and had red discolouration of urine from day 28 onwards. This animal showed piloerection from day 42. As the general condition of this animal was very poor on day 49, the animal was sacrificed. The reason for the bad clinical condition was reported to be 'predominantly spontaneously occurring kidney lesions' and was not considered treatment-related. Red discolouration of urine was also seen in a LD male from day 77. A HD male had respiration sounds (moderate, pulmonic) starting from day 84. One control female had a palpable mass in the skin with missing vaginal opening from day 63. One MD female showed exophthalmia from day 77 and one HD female showed a tooth anomaly from day 28 onwards. The study considered all these findings as incidental. Rearing (observed in the functional battery conducted on day 80 or 82) was higher in MD and HD male groups by 105% and 130%, respectively but it is not clear whether this was a transient change. Body weights were slightly lower in HD males from day 35 (~3% compared to the control group; terminal body weight was lower by 3.7%) and the changes were not statistically significant. There were no treatment-related changes in locomotor activity, food consumption (or efficiency) or ophthalmology.

At haematological examination (day 94), lower WBC counts were seen in all treated male groups (by 21%, 15% and 21% in the LD, MD and HD groups, respectively) but the changes were not statistically significant or dose-dependent and were not seen in female groups. Prothrombin time was not affected by treatment.

Changes in clinical chemistry (day 94) included lower bilirubin (total) levels in the HD male group (-12%, statistically significant) and lower triglycerides in all treated male groups (by 14%, 23% and 23% in the LD, MD and HD groups, respectively; not statistically significant). Blood magnesium levels were significantly lower in the HD female group (by 14%).

At urinalysis on day 88, urinary bilirubin levels were found to be higher ($\geq$25 µmol/L in 3 HD animals; the levels were $\leq$9 µmol/L in all the other animals, including the control group; urobilinogen was not affected) in 3/9 HD males.

There were no treatment-related changes in sperm parameters.

In the HD group, slightly higher (but statistically significant) weights of liver (relative weights: +7% in males and +10% in females) and testis (relative weights by 9%) were seen. In treated females, lower weights of spleen in the MD group and higher weights of heart in the LD group were seen but the changes were unlikely to be treatment-related because of the absence of dose-response relationship or histological changes.

Gross pathological and histological lesions were seen mainly in the animal that was killed on day 49. The gross pathological lesions included dark red discolouration of the lungs, severely enlarged kidneys that revealed a granular surface, slight dilation of the ureters and moderately reduced organ sizes of seminal vesicles and prostate gland. The study report stated that the gross lesions in the animal were responsible for its premature sacrifice. The study interpreted the changes to be of spontaneous origin.

In the animal which was killed prior to scheduled sacrifice, histological changes were seen in the testes (altered spermatogenesis, with degeneration and necrosis of germ cells and focal tubular atrophy, unilateral), seminal vesicle (atrophy), coagulation glands (atrophy), prostate (hypoplasia), heart (focal necrosis) and kidneys (lymphoid cell infiltration). Changes in the other animals or other organs (including the liver, spleen or uterus) were either unremarkable or not dose-related.

All the changes in the study, except changes in the liver weights in the HD group, were attributed by the study to be incidental findings and not treatment-related. The changes in the liver weights were not considered toxicologically relevant since there were no accompanying changes in histology. It was noted that the magnitude of the liver weight changes was small (≤10%) and hence the changes may not even be biologically relevant.

Clinical chemistry changes included lower levels of total bilirubin in serum and higher levels of bilirubin in the urine. It was noted that the biotransformation of unconjugated bilirubin (which is albumin-bound in plasma) takes place in the liver. The accelerated elimination (associated with decreased plasma bilirubin) of serum bilirubin showed that the hepatic bilirubin metabolism was increased. Also, the metabolism of xenobiotics is often associated with bilirubin metabolism, the decreased serum bilirubin concentration was considered to be a sign of metabolic adaptation to exposure to the test material.

The no observed adverse effect level (NOAEL) was 15000 ppm (1249 or 1452 mg/kg/day in males and females, respectively; mean: 1350 mg/kg/day).

REPRODUCTIVE TOXICITY

> 2-(4-Diethylamino-2-hydroxybenzoyl)-benzoesäurehexylester - Prenatal developmental toxicity study in Wistar rats. Oral administration (gavage). BASF Aktiengesellschaft, Germany; project no. 30R0408/99112; K. Schilling; 6 June 2001; GLP/QA-yes.

Diethylamino hydroxybenzoyl hexyl benzoate was assessed for its potential teratogenic activity in groups (25/dose) of mated female Wistar rats at dose levels of 0 (vehicle only), 40, 200 or

1000 mg/kg/day orally. The test substance (in olive oil) was administered by gavage from gestation day 6 through to gestation day 19. 'Due to technical reasons', the study was carried out in 3 'sections' (group/groups for each section were not identified): an interval of 1-2 days existed between the treatment starting dates for each dose group (presumably to avoid sacrificing all the dams on the same day at the end of treatment). The animals were sacrificed on gestation day 20 and their uterine contents were removed for investigation. All the fetuses were examined for external abnormalities while one half of fetuses per dam were subjected to visceral examination and the other half for skeletal examination. In maternal animals, the parameters followed during the course of the study included survival, body weight, food consumption, clinical signs of toxicity and necropsy.

Maternal parameters: There were no deaths. Reproductive data showed that 25, 23, 23 and 22 of the mated females from the control, LD, MD and HD groups, respectively, were found to be pregnant. Although there were slightly fewer pregnant females in the treated groups this difference had nothing to do with treatment as mating occurred well before administration of diethylamino hydroxybenzoyl hexyl benzoate started. In the MD and HD groups (25 and 21 rats, respectively, including non-pregnant animals), transient salivation was seen during the entire treatment period (except on the first day), lasting only for a few minutes after each administration of the test substance (no salivation in the control or LD group). Food consumption was lower in the HD group during the initial half of the treatment period (by 9-15% on gestation days 6-13, statistically significant; the overall mean food consumption during the entire treatment period was lower by ~7% but was not statistically significant). Body weights in the HD group were not significantly different to the control group (295.1 and 289.2 g in the control and HD groups, respectively on gestation day 20). However, after adjusting for gravid uterine weights, body weight gain in the HD group was 11% lower than that of the control group. In the MD group, food consumption was slightly but significantly lower on gestation days 6-8 (by 6%). Body weight changes were not remarkable in this group. At necropsy, one HD animal was reported to have bilateral 'hydrometra' (the animal was not pregnant). There were no other treatment-related changes at necropsy.

Uterine parameters: The mean number (per pregnant female) of corpora lutea, implantations, live fetuses, dead fetuses (nil), placental weights, and sex ratio and weight of fetuses were not affected by treatment. The incidence of post-implantation loss was higher in the MD and HD groups when compared to the control group (% incidence: 4.8, 5.7, 9.4 and 9.6 in the control, LD, MD and HD groups, respectively) but the incidences were within the historical control (5.5-11.5%) range. One HD dam had complete resorption. Although complete resorption was not seen in the other groups, complete resorption is known to occur at a low incidence in the animal strain used in this study (historical control: 2.1% dams had complete resorption ie. 4 dams out of 190) and hence the resorption seen in the HD group may not be treatment-related.

External, visceral and skeletal examinations of the fetuses: External examination of the fetuses revealed anasarca (general oedema) in one fetus (out of 248 fetuses; 0.4%) and unilateral anophthalmia in another fetus, both in the LD group. The historical control data did not list any anophthalmia but has included a low incidence of anasarca (0.1%). There were no treatment-related effects seen at visceral examination. Skeletal malformations included misshapen scapula (cartilage present) in one MD fetus but the incidence (0.8%) was within historical control range

(up to 2.7%) and hence it is not likely to be treatment-related. A few other skeletal malformations were seen in the treated groups, with incidences comparable to those of the control group. There were several skeletal variations or other changes (unclassified changes) in the treatment groups but most of these changes (some of these are shown in the table below) were either comparable to concurrent control or historical control, or did not show dose-dependent changes. However, incidences of wavy rib in the MD and HD groups, and notched cartilage between basisphenoid and basioccipital bones and incomplete ossification of lumbar arch in the HD group were likely to be treatment-related since the incidences of these changes were clearly higher than the concurrent or historical control.

% Incidence of skeletal variations and unclassified cartilage changes

	0	**LD**	**MD**	**HD**	***Historical control range***
Wavy rib	5.8	2.3	8.3	14.7	*0.9-5.6*
Notched cartilage between basisphenoid and basioccipital bones	1.5	2.3	1.7	4.3	*0-1.8*
Incomplete ossification of lumbar arch	0.7	0	0.8	3.4	*0-0.9*
Unossified hyoid	0.7	0	2.5	0.9	*0-4.5*
Incomplete ossification of hyoid	0.7	0.8	3.3	0	*0-0.9*
Extra ossification site between parietal and interparietal bones	0	0	0.8	0	*Not stated*
Incomplete ossification of frontal	0	0.8	0	0.9	*0-3.6*
Bipartite ossification of supraoccipital	0	0	0.8	0	*0-0.9*
Incomplete ossification of thoracic centrum	2.2	5.5	10.8	4.3	*0-6.5*
Unilateral ossification of thoracic centrum	0	0	0.8	0	*Not stated*
Dumbbell ossification of lumbar centrum	0	0	0.8	0	*0-1.9*
Fused sacral centrum and arch	2.9	2.3	4.2	5.2	*0-4.7*
Unossified sternebra	12	20	22	10	*4.7-29.1*
Incomplete ossification of sternebra	59	70	71	64	*32.1-69.2*
Supernumerary rib (14th); cartilage present	1.5	2.3	4.2	2.6	*Not stated*
Temporale hole(s)	0	0	1.7	0	*0-0.9*$^{\#}$
Fused cervical arch cartilage	0	1.6	3.3	3.4	*0-2.7*
Dumbbell-shaped cartilage of cervical centrum	0	0	0.8	0	*Not stated*
Dumbbell-shaped cartilage of thoracic centrum	0.7	1.6	1.7	0	*0-1.9*
Notched manubrium	5.8	10.2	6.7	7.8	*0-11.6*
Cartilaginous parts of ribs displaced	*0*	*0.8*	*0*	*0.9*	*0-0.9*

n = 116-137 fetuses/group except historical control (n = 439). $^{\#}$ Incidence for temporale fissure.

Overall, it was apparent that diethylamino hydroxybenzoyl hexyl benzoate at 200 and 1000 mg/kg/day administered orally during gestation caused maternal toxicity (salivation and reduced food consumption at 200 and 1000 mg/kg/day; reduced body weight gain at 1000 mg/kg/day) and had adverse effects on the development of the foetuses (skeletal variations). The no-effect level for maternal toxicity and embryofetal toxicity was 40 mg/kg/day.

Please note that the study report determined a no observed adverse effect level (NOAEL) of 200 mg/kg/day for maternal toxicity (transient salivation and reduced food consumption; did not consider salivation at 200 mg/kg as treatment-related: see below) and 1000 mg/kg/day for prenatal developmental toxicity (embryofetal toxicity; the report stated that there were no treatment-related effects on gestational parameters and no signs of prenatal developmental toxicity). The study report considered transient salivation at 1000 mg/kg/day as a maternotoxic effect but salivation at 200 mg/kg/day was considered as a non-maternotoxic effect and was reported to be most probably related to irritating or bad-tasting properties of the test substance (please note that the test substance was given by gavage and not in diet).

CARCINOGENICITY

No data were submitted addressing the issue of potential carcinogenicity. A rationale for absence of carcinogenicity data was submitted, which is present and discussed in the summary/assessment section of this report.

Based on an *in vitro* dermal absorption study. There were technical problems in the study.
Based on a photosensitisation study in a low number of animals.
US EPA (1995). Federal Register: Inert ingredients in pesticide products; reclassification of certain List 3 inert ingredients to List 4B. July 7, 1995. Federal Register: Volume 60, no. 130.
http://www.epa.gov/EPA-PEST/1995/July/Day-07/pr-331.html
18000 mg product x 10% excipient x 1% dermal absorption ÷ 50 kg bw (for females) = 0.36 mg/kg/day of diethylamino hydroxybenzoyl hexyl benzoate. The NOEL was 40 mg/kg/day (oral) in the rat developmental toxicity study. Hence the safety margin (margin of exposure; 40 mg/kg/day NOEL ÷ 0.36 mg/kg/day exposure) is only 111.

The 200 μg/cm^2 concentration was reported to be a dose level reflecting application conditions according to the recommendations of the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) intended for consumers.
Wistar (SPF)/Crl:WI (GLX/BR/HAN)IGS BR rats
Harlan (Hsd Proc: DH) SPF guinea pigs.
0 = no visible change; 1 = discrete or patchy erythema; 2 = moderate or confluent erythema; 3 = intense erythema and swelling. In addition, E = swelling; + = incrustation, partially open.
Crl: WI (GLX/BRL/HAN) IGS BR strain.
The doses were selected from a preliminary study in 3 animals/sex. The animals were given 5000 and 15000 ppm of the test substance for 2 weeks. According to the study report, there were no signs of toxicity in the parameters tested (clinical signs, food and water consumption, body weight and gross pathology).

CRL:WI (GLX/BRL/HAN) IGS BR strain.
Presence of sperm in the vaginal smear was taken as gestation (post coitum) day 0 and the following day was gestation day 1.

OTC MEDICINES EVALUATION SECTION

ASSESSMENT OF NEW TOPICAL ACTIVE

Tinosorb S – UV filter

Sponsor:	Ciba Speciality Chemicals
File Number:	2004/022962
TGAIN:	197373
Report Date:	July 2004
Evaluator:	s22 [redacted]

IDENTITY

INCI:	Bis-ethylhexyloxyphenol methoxyphenol triazine (MEBT)
AAN:	
CAS No.:	187393-00-6
Chemical Name:	2,4-Bis-{[4-(2-ethyl-hexyloxy)-2-hydroxy]-phenyl}-6-(4-methoxyphenyl)-(1,3,5)-triazine
Trade Name:	Tinosorb S
Company Code:	CGF-C1607
Drug Class:	UV filter
Empirical Formula:	$C_{38}H_{49}N_3O_5$
Molecular Weight:	627.80 g/mol
Physical Properties:	Melting point 80^0C, boiling point >400^0C
Density:	1170 kg/m^3
Water Solubility:	<14 µg/L at 20^0C
Partition coefficient:	9 (logPow)
Structure:	

SUMMARY OF TOXICOLOGICAL FINDINGS

Kinetic data single oral dose in rats	0.1-0.2% absorbed
Kinetic data single dermal dose in rats	0.1-0.4% absorbed
In vitro human percutaneous assay	0.14% in receptor fluid

(Note: amount absorbed is percentage of dose applied/ingested)

Acute dermal toxicity in rats	LD50 >2000 mg/kg
Acute oral toxicity in rats	LD50 >2000 mg/kg
Primary skin irritation in rabbits	Non-irritating
Primary eye irritation in rabbits	Non-irritating
Skin sensitisation (maximisation test) in guinea pigs	Not sensitising
Phototoxicity in guinea pigs	Not phototoxic
Photoallergenicity in guinea pigs	Not photoallergenic
Phototoxicity in humans	Not phototoxic
Photoallergenicity in humans	Not photoallergenic
14-day oral range finding study in rats	NEL 2000 mg/kg/day
90-day oral toxicity study in rats	NEL 1000 mg/kg/day
14-day dermal range finding study in rats	NEL 1000 mg/kg/day
90-day dermal toxicity study in rats	NEL 1000 mg/kg/day
Range finding developmental study in rats	NEL 1000 mg/kg/day
Development toxicity study in rats	NEL 1000 mg/kg/day

(Note: the NEL values in the above studies were the highest doses used in each of these studies).

Genotoxicity Ames & E.coli assays	Negative
Photomutagenicity/E. coli & S. typhimurium	Negative
Chromosomal aberration in CHO cells	Negative
Photomutagenicity/chromosomal aberration in CHO cells	Negative
In vivo mouse micronucleus test	Negative
Androgen receptor binding assay (*in vitro*)	Negative
Oestrogen receptor binding assay (*in vitro*)	Negative
Uterotrophic assay in rats treated orally	Negative

INTRODUCTION

Characteristics of Tinosorb S

It was noted that because of the very low water solubility and a partition coefficient of 9 that bio-accumulation would not be anticipated. A study in carp supported this contention that Tinosorb S does not bio-accumulate in a species of fish.

A study (Ciba; ref. No. GZ5.212; B. Herzog; 1998) of photostability of Tinosorb S measured its recovery after application of different doses of UV light/radiation. Doses of UV radiation were varied between 0 and 50 MED (minimal erythemal dose) followed by analysis using UV spectroscopic and HPLC methods. After dosing at the highest exposure rating of 50 MED recovery of Tinosorb S was in the range of 95% to >98% depending on the method of analysis. This indicated that Tinosorb S was very photostable under extreme conditions of UV exposure.

UV light absorption spectrum

Data generated using a spectrometer showed that Tinosorb S complied with the current Australian Standard (Standards Australia:- AS/NZ 2604:1998) for classification as providing UVA protection when used at a concentration of 2% in a product. This standard indicates that UVA-protection is recognised when a sunscreen preparation transmits between 320-360 nm less than 10% of the incoming relevant UV radiation.

An often used parameter known as critical wavelength is defined as the wavelength at which the extinction (blocking) capacity reaches 90% of its overall extinction capacity. The higher the critical wavelength of a sunscreen, the better is the UVA protection performance. Tinosorb S had a critical wavelength (λ_c) of 370 nm.

The UVA to UVB ratio is based on a comparison of the areas under the extinction curves over wavelength ranges of 320 – 360 nm (UVA) to 290 – 320nm (UVB). The UVA/UVB ratio for Tinosorb S was 0.73.

International status

Documented registration status of Tinosorb S provided by the sponsor indicates approval (since March 2000 in Europe and South America) granted for use in Europe (included in Annex VII of the EU Cosmetics Directive 76/768/EEC), French Guyana, Switzerland, Brazil, Argentina, Paraguay, Bolivia, Uruguay, China and South Africa. Available for use in products in Hong Kong, Singapore and Korea, where use is permitted provided no therapeutic claims are made. In all countries where Tinosorb S has undergone a registration process the concentration approved for use in sunscreen products is up to 10% active ingredient.

It was noted that by the end of 2004, Tinosorb S will have been incorporated in about 100 million SKU's (stock keeping units) of sunscreens or day creams, mainly in Europe and Brazil. However, there was no indication that an analysis (data collection) of adverse events in humans was conducted for Tinosorb S.

ASSESSMENT

Kinetic findings

The data submitted included ADME (absorption, distribution, metabolism and excretion) studies for the oral and dermal routes in rats, as well as an in vitro percutaneous absorption study using human skin. An ADME study using the IV route in rats was not carried out, which means absolute bioavailability data could not be generated. Blood samples were taken at intervals (day 8, and weeks 6 and 13) in the 13 weeks dermal toxicity study for the determination of plasma levels of Tinosorb S. However, measurement of plasma levels of Tinosorb S in the 13 weeks oral toxicity study in rats were not conducted.

After oral dosing in the rat, systemic exposure to Tinosorb S was found to be very low (based a urinary data) at around 0.1-0.2% of the administered dose. Systemic metabolite profiling was not

possible due to the extremely low amount found in the plasma and passed in the urine, while unchanged Tinosorb S was the only peak found in the faeces of rats treated via the oral route. This shows that Tinosorb S was stable under the conditions experienced in the GIT. It was apparent that repeat-dose oral toxicity studies in rats (and probably other animal species) were unlikely to experience significant systemic exposure to Tinosorb S due to the extremely low absorption from the GIT.

After topical (dermal) dosing in the rat, systemic exposure to Tinosorb S was found to be very low (based on urinary data) at around 0.1-0.4% of the administered dose. Metabolite profiling was not possible due to the extremely low amounts of radioactivity found in the plasma or passed in the urine. It was apparent that repeat-dose topical (dermal) toxicity studies in rats (and probably other animal species) were unlikely to experience significant systemic exposure to Tinosorb S due to the very low absorption through the skin.

Kinetic data collected from the repeat-dose dermal toxicity in rats showed that accumulation of the test material was extremely unlikely. Plasma levels of Tinosorb S at sampling points of days 8, 37 and 91 were either slightly above (4.81-10.00 ng/mL) the limit of detection (2 ng/mL) or not detectable. Higher plasma levels were observed at day 37, while plasma levels of zero (below detection limit) and 6.35 ng/mL were seen at day 91 in females and males, respectively.

In an *in vitro* percutaneous absorption study with human skin, applied Tinosorb S hardly penetrated the skin, with 0.14% of the applied dose recovered in the receptor fluid. The permeation rate was calculated to be approximately 0.004 μg/cm^2/hour, which was extrapolated to represent 0.08% permeation over a 24-hour period.

Overall, the degree of potential systemic exposure following dermal and oral administration of Tinosorb S in the rat was very similar (0.1-0.2% vs 0.1-0.4%), while the permeation of Tinosorb S through human skin (*in vitro*) was also similar or marginally less.

The sponsor provided an estimate of the margin of safety, which was calculated as follows:- Parameters used in the calculation were body weight of adult (60 kg), body surface area (18000 cm^2), Tinosorb S (10%) applied (2 mg/cm^2 = 3.6 g) and skin absorption of active (0.4%) based on species/study used to generate NEL. The figures led to a value for systemic exposure of 14.4 mg or 0.24 mg/kg/day (60 kg adult). The sponsor generated safety factor was NEL/systemic exposure (1000/0.24), which was found to be 4167.

This is a simplification of the situation, which would be corrected if the systemic exposure associated with the NEL of 1000 mg/kg/day was compared to the estimated systemic exposure achieved by applying Tinosorb S (in sunscreen) to the skin. This would be a better approximation of a comparison of AUC values, which is the desired approach to relate exposures. Therefore, the systemic exposure associated with the dermal NEL of 1000 mg/kg/day would be 0.4% of 1000 = 4 mg/kg/day, which is still 16.6 times the anticipated systemic exposure when applying Tinosorb S to human skin. It must be remembered that there was no evidence of toxicity at a dose of 1000 mg/kg/day (highest dose used) in rats, which means the actual threshold dose for toxicity could be considerably higher. Also, the safety factor of 16.6 would be higher (safer) when applying the observed value (<0.1%) for absorption through

human skin and not the value for dermal absorption in animals, which was approximately 4 fold greater. Therefore, a conservative but more accurate estimate for a safety margin would be 4 x 16.6 = 66.4.

Interaction potential

It was stated that Tinosorb S has been on the market in the EU and in Latin American countries for several years (4 actually). Tinosorb S has been formulated with a number of other UV filters in marketplace products. It was noted that Tinosorb S is compatible with all other approved UV absorbers used in Australia, Europe, USA and Japan. UV filters mentioned individually as having been formulated with include ethylhexylmethoxycinnamate (EHMC), ethylhexyltriazone (EHT), butylmethoxydibenzoylmethane (BMBM), titanium dioxide and zinc oxide. BMBM is known to be photo-labile, but formulated with Tinosorb S it was stabilised. A study by Herzog & Sommer (XXIst IFSCC International Congress 200, Berlin; Proceeding) supported the previous statement, finding that Tinosorb S did stabilise BMBM under conditions of irradiation.

Attachment 1 provides tabulated information on products containing Tinosorb S and other UV absorbers.

Local tolerance

In studies in animals, Tinosorb S was not a primary skin (100% solution) or eye irritant in the rabbit. In a repeat-application study, the clipped skin of guinea pigs were exposed to Tinosorb S (up to 10%) daily (5 days/week) over a 2 weeks period and found to be minimally reactive (minimal irritation occurred). Contact hypersensitivity (maximisation test) to Tinosorb S (up to 30%) was assessed in a guinea pig assay, which found that the test material was not a skin sensitiser under conditions of heightened responsiveness.

Tinosorb S (up to 30%) was assessed for potential phototoxicity in an animal model using guinea pigs and found to be negative with no evidence of reaction following exposure to UV radiation. A photoallergenicity assay (with maximisation conditions) in guinea pigs found that Tinosorb S (up to 30%) did not induce a photoallergic response in guinea pigs.

Clinical studies were provided that examined the potential of Tinosorb S to induce either phototoxicity or photoallergenicity. In the phototoxicity assay, Tinosorb S was applied too human skin in a lotion at a concentration of 10% prior to UV irradiation. Tinosorb S was less reactive than placebo and saline solutions prior to and after irradiation indicating that it was not phototoxic. In a repeat insult patch test assessing photoallergic reactivity, an induction period of 3 weeks with Tinosorb S (10% in lotion) preceded rest (2 weeks) and challenge periods. Appropriate UV irradiation was incorporated into the induction and challenge sections of the study. Tinosorb S was less reactive than placebo and saline solutions prior to (during induction) and after challenge in the presence of UV radiation indicating that it was not photoallergenic in humans.

It is important to note that negative results in skin sensitisation and photoallergenicity studies in humans and guinea pigs indicates negligible interaction of the test material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and potentially, to DNA.

General toxicity

Acute oral and dermal toxicity studies in rats showed that Tinosorb S has low acute toxicity, with LD50 values of >2 g/kg for both routes. There was no evidence of acute toxicity, which is not surprising considering the expected low systemic exposure for both routes.

Repeat-dose dermal toxicity was examined in a 2 weeks dose range finding study and a 13 weeks repeat-dose toxicity study in rats. The dose range-finding study identified that a topical dose of up to 1000 mg/kg/day (in PEG 400) had no adverse effect on survival and was well tolerated. In the main dermal study, repeated daily dosing over a 13-week period with up to 1000 mg/kg/day Tinosorb S had no discernible treatment-related adverse effects. There were incidences of minor skin reactions, but these were seen in all groups including controls, and they did not occur in a dose-related manner. Furthermore, observed skin reactions were limited to the first 10 days of treatment and animals with collars on (to limit licking of application sites) showed no signs of erythema and minimal evidence of any other skin response. It would appear that direct application of Tinosorb S to the skin of rats (in PEG 400) at doses up to 1000 mg/kg/day was without apparent adverse activity. It was suggested that the observed minimal effects were related to the presence of residue or the effect of licking at the application site. Parameters measured during the course of the study did not display any trends that could be identified as treatment–related. Plasma levels of the test material were measured in samples taken at days 8, 37 and 91 of dosing, with results from this analysis presented in the kinetic of this report. A NEL of 1000 mg/kg/day was estimated for dermal/topical application of Tinosorb S in the rat.

Repeat-dose oral toxicity was examined in a 2 weeks dose range finding study (using up to 2000 mg/kg/day) and a 13 weeks repeat-dose toxicity study in rats. In the dose range-finding study, there was no evidence of toxicity up to the highest used, while a clinical sign of pale faeces was thought to be related to large amounts of ingested test material. A dose range of 100, 500 and 1000 mg/kg/day was selected for the main study. In the 13 weeks main study, there were a number of fluctuations across a range of parameters but these were considered not to be treatment-related for the following reasons. They lacked a dose-relationship, occurred only in one sex, were found to be within historical control ranges for that value and/or identified as an isolated finding, which was not associated with relevant tissue/organ changes (eg liver enzymes↑ – liver weight? – liver histology?). Overall, a NEL of 1000 mg/kg/day was estimated for Tinosorb S administered orally to rats.

Results from these oral and dermal toxicity studies indicated that Tinosorb S did not display toxicity up to the maximum dose of 1000 mg/kg/day. The fact that no toxicity was observed questions the value of these studies since target organs/systems of toxicity and threshold levels of Tinbsorb S were not evident with doses not pushed high enough? However, the use of higher doses (oral or dermal) may be counter-productive based on the kinetic information that showed such low exposure to Tinosorb S using these routes. Importantly, the proposed route of use in humans was covered by the inclusion of repeat-dose dermal toxicity studies and appropriate kinetic data on the animal species used in these studies.

Reproductive toxicity

The potential reproductive toxicity of Tinosorb S was examined in a prenatal toxicity study (2 studies presented, dose-ranging and main assay) and a uterotrophic assay in rats. In the prenatal assay, the test agent (up to 1000 mg/kg/day) was administered orally during the period (days 6-17 of gestation) of organogenesis to pregnant female and could more accurately be described as a developmental (teratogenicity) study. This study found that Tinosorb S was not teratogenic at doses up to 1000 mg/kg/day delivered during organogenesis. This extent of investigation into the reproductive toxicity of a new substance would be considered inadequate by DSEB standards, with fertility and peri/post natal studies required, as well as a second developmental study in a species other than a rodent (usually a rabbit). However, studies investigating these reproductive end-points using the oral route would be futile due to extremely low absorption from the GIT and very low systemic exposure.

The sponsor acknowledged the absence of a full range of reproductive toxicity studies. A rationale for the absence was attributed to results from the sub-chronic study in rats (oral and dermal) where Tinosorb S was not anticipated to have adverse effects on fertility and reproduction. Their conclusion was based on an absence of adverse organ weight changes and pathology findings for reproductive organs and tissues following both oral and dermal administration of Tinosorb S (up to 1000 mg/kg/day) for 13 weeks.

The sponsor also referred to additional studies examining issues related to potential adverse effects on reproductive tissues. These studies included a rat uterotrophic assay and androgen and oestrogen competitive binding assays. These assays demonstrated that Tinosorb S did not interfere with normal uterotrophic maturation in rats and it did not bind (or interfere with binding of other agents) to androgen or oestrogen receptors derived from rat prostate or uteri. These results indicate that Tinosorb S would be unlikely to possess oestrogenic or androgenic activity.

Genotoxicity

Genotoxicity was examined in a series of *in vitro* assays using both bacterial and mammalian cell systems, as well as *in vivo* in mice. In these studies, Tinosorb S was shown not to be a mutagen in bacterial reverse mutation assays and in an *in vitro* chromosomal aberration assay in Chinese hamster V79 cells. The potential for photo-mutagenicity was also examined in bacterial and mammalian cell systems *in vitro*. Tinosorb S was shown not to be a photo-mutagen in bacterial reverse mutation assays and in an *in vitro* chromosomal aberration assay in Chinese hamster V79 cells that incorporated exposure to UV radiation.

The results from these assays may have been affected by an inability of Tinosorb S to interact with appropriate organelles in the cell cultures. The size of the Tinosorb S molecule (MW 627) may have had an influence on its ability to penetrate cells to interact with genetic material and therefore affect the validity of the assays. If penetration as based only on MW, then it is possible penetration (and intracellular exposure to Tinosorb S) occurred since a number of antibiotics are around this MW and others exceed it by up to 3-fold (vancomycin, MW 1449). However, cell penetration is based on more factors than just size and exact understanding as to whether Tinosorb S penetrates into bacterial and mammalian cells *in vitro* is not available.

An *in vivo* bone marrow micronucleus assay in mice using intraperitoneal administration of Tinosorb S was submitted, which showed that Tinosorb S did not induce an increase in the incidence of micronuclei. Administration of Tinosorb S via the intraperitoneal (IP) route meant that the limitation of absorption/exposure seen with oral and dermal routes was not an influence on the result. However, there were no data on systemic distribution (kinetics) of Tinosorb S following IP injection, therefore the possible significance of this difference in route of administration was not assessable/quantifiable.

Overall, Tinosorb S was not a mutagen or photo-mutagen in a series of *in vitro* assays in bacterial and mammalian cell systems, and it was not a mutagen in an *in vivo* assay in mice. The genotoxicity data package submitted for Tinosorb S was similar to that required by DSEB for assessment of a new chemical entity.

Carcinogenicity

A carcinogenicity bioassay in a rodent model was not included in this submission. However, the sponsor provided a rationale (attachment 3) for not conducting carcinogenicity testing for Tinosorb S, which was based on the findings from the submitted studies and known characteristics of identified human carcinogens. References cited in the rationale have been checked. Furthermore, these have been identified as the same 24 references previously presented in the rationale in support of a similar exemption from the need to conduct a carcinogenicity study for Tinosorb M.

The basis for the sponsor request for not conducting carcinogenicity testing can be summarised in the following points:

1.Tinosorb S was not photogenotoxic or genotoxic (*in vitro* and *in vivo*) in a series of assays in bacteria and mammalian cell systems measuring various end points (reverse mutation, chromosomal aberration).
2.Tinosorb S did not induce dermal acanthosis or other hyperproliferative dermal changes in rats after repeated dermal treatment.
3.Tinosorb S is stable to UV light and does not generate photo-metabolites or breakdown products.
4.Tinosorb S is not a skin contact allergen in the presence or absence of UV irradiation and it does not cause phototoxic skin reactions.
5.Tinosorb S does not have the profile of known skin carcinogens and will not be a dermal carcinogen.
6.The sponsor concludes by suggesting that Tinosorb S should not need to undergo dermal carcinogenicity testing because of the preceding (1-5) reasons and would be safe for use in listable sunscreen products in Australia.

Tinosorb S did not generate a positive response in any of the genotoxicity or photogenotoxicity assays (*in vitro* and *in vivo*) provided, indicating that it did not behave as a genotoxic agent. Furthermore, it is important to note that negative results in skin sensitisation and photoallergenicity studies in humans and guinea pigs indicates negligible interaction of the test

material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and potentially, to DNA.

The sponsor acknowledged the existence of non-genotoxic mechanisms of skin cancer and addressed the issue of whether such mechanisms could be relevant to Tinosorb S. The sponsor noted that skin tumour promoters consistently cause sustained dermal hyperplasia (acanthosis) in mouse skin models. Tinosorb S did not cause hyperkeratosis or acanthosis in the dermal studies presented in this submission, but it was indicated (referenced) that rat skin would not be expected to response to non-genotoxic mechanisms of tumour induction.

It was proposed that chronic skin irritation and inflammation could also be relevant to topically applied substances having a potentially adverse effect leading to skin cancer. However, studies in rats have shown that agents causing identified macroscopic and microscopic signs of chronic irritation and inflammation did not cause skin cancer. These non-genotoxic causes (mentioned above) of skin change did not lead to skin carcinogenesis, which highlights the fact that indirect mechanisms of carcinogenicity are not simple processes. However, Tinosorb S was not a dermal irritant at doses up to 2000 mg/kg/day (2-week study) and there was no evidence of chronic irritation or inflammation in a longer study with levels of up to 1000 mg/kg/day. The sponsor concluded that Tinosorb S does not have the profile of a non-genotoxic skin carcinogen, but as stated above the process (skin carcinogenesis) of identifying skin carcinogens is not a simple one.

RECOMMENDATION

The sponsor has submitted a package of data containing studies on Tinosorb S that conform to GLP standards and OECD or ICH guidelines. Tinosorb S is a UV filter that has been on the accepted list of UV filters in the EU since March 2000 at concentrations of up to 10% in products. Tinosorb S has also been approved for use in numerous South American countries, South Africa and China at concentrations up to 10%.

The features of Tinosorb S are:-

Systemic exposure in rats following either administration via the oral or dermal route was very low (<0.5%), and *in vitro* percutaneous penetration through human skin was 0.14%.

Local tolerance studies found that Tinosorb S was not a skin or eye irritant, skin sensitiser and did not cause skin phototoxicity or photoallergy (in humans and animals).

No definable toxicity in animal studies (developmental, repeat-dose oral or dermal) at a dose of up to 1000 mg/kg/day. Data on the reproductive toxicity profile of Tinosorb S were limited primarily to developmental studies (main and dose range-finding) in rats, which was supplemented with data showing Tinosorb S did not interfere with androgen and oestrogen receptors and was not uterotrophic in the rat.

Tinosorb S was not a genotoxic agent in a series of *in vitro* and *in vivo* assays. A rationale for the absence of a dermal carcinogenicity study (rat bio-assay) was submitted, which was based on toxicity (genotoxicity or local tolerance) profile and no structural alert features (comparison with known carcinogens). It would appear that the likelihood of Tinosorb S being carcinogenic was low or negligible based on the information provided.

In the data provided, Tinosorb S displayed a lack of definable toxicity at a systemic exposure likely to be significantly greater than anticipated to occur in humans following dermal application in sunscreen products. On this basis, Tinosorb S (UV filter) appears to be acceptable for use in listed/registered sunscreen products at concentrations up to 10%.

The advice/opinion of the committee is requested

DATA EVALUATION

Clinical studies

Kinetic data

In vitro human skin penetration and distribution of the UV absorber CGF-C1607 [Tinosorb S] (An-eX Analytical Services Ltd; report no. CSC/8/98; A. Watkinson; 1998; GLP/QA-yes).

In this study, the in vitro skin penetration and distribution of Tinosorb S was examined after a 24 hour application period to excised human skin. This assay was conducted in accordance with the COLIPA guidelines for percutaneous penetration of cosmetic ingredients (1995). A formulation containing 10% Tinosorb S was applied to human epidermal skin membranes mounted in Franz type diffusion cells at a target dose of 2 mg/cm^2. The receptor cell medium was modified (6% Oleth 20 in phosphate buffered saline) due to very low solubility of Tinosorb S in normal medium. It was noted that Oleth 20 in phosphate buffered saline has been shown not to effect the permeability of human skin and to be a good solubilising agent for lipophilic compounds. A total of 12 skin samples were used in this study.

Recorded data indicated that only 6/12 of the skin samples used allowed penetration of the test material through into the receptor fluid. Overall, penetration through the skin was very low at 0.04±0.02 μg/cm2, which represented 0.02% of the applied dose after 24 hours. It was noted that extrapolation of permeation profile at various times was difficult due to the very low levels of penetration and plateauing effect that occurred. Assessment of distribution of the applied test material showed the majority was recovered (>80%) either on the skin surface or in the first 3 tape strips. Remaining material was recovered in the tape strips 4-20 (10.2% of applied dose), the remaining sample of skin (7.3%) or in the receptor phase (<0.1%). The skin permeation rate was calculated to be approximately 0.004 μg/cm^2/hour. For a 24-hour period the permeation was calculated to be 0.08 μg/cm^2 or 0.08% of the applied test material. Recovery of the test material was 99%.

Local tolerance

Evaluation of phototoxicity in humans (Hill Top Research Inc; Project no. 100243A; J. Plautz; 1998; GCP/QA-yes).

This study carried out an assessment of the potential phototoxicity of Tinosorb S (in lotion at 10%) after a single application to the skin of humans (26 volunteers, 22 females and 4 males). A single application (24 hour contact time) of duplicate patches was made to naïve sites on the paraspinal region of the back. One of the duplicate patch sites was irradiated with UV (A & B) radiation, while the other was used to evaluate primary irritation potential, as well as act as a non-irradiated control.

Materials used in this study included Tinosorb S (CGF-1607), lotion placebo and saline solution; these materials were supplied by the sponsor Ciba Specialty Chemicals Inc. These materials were

applied neat in a volume of 0.2 mL using a Hill Top chamber, which was semi-occluded. Patches (Hill Top chamber) were placed in position and removed by a laboratory technician. Contact time was 24 hours after which the sites were cleaned free of residual material prior to irradiation immediately after removal of the patches. Irradiation was based on the MED (minimum erythema dose), which was determined for each subject prior to exposure. Doses of UVA (16 J/cm^2) and UVB (0.75 J/cm^2) were those found to be suitable. All test sites were evaluated at approximately 1, 24, 48 and 72 hours after UV irradiation and patch removal.

There was one reported adverse event, which occurred in subject 02 on April 14 1998. This subject reported a moderate rash on the waist area of the abdomen. The subject felt that it may have been related to the use of a deodorant soap, since she had a history of reactions with deodorant soaps. The rash improved after topical application of Cortiad (topical anti-inflammatory product). The symptoms subsided on use of Cortaid and were no longer present by April 25. It was considered that this reaction was not due to the test material, based on its location, a history of reaction to deodorant soaps and being an isolated finding.

Tabulated data did not accurately reflect the individual findings (presented in appendix II) for the placebo/irradiated group, while all other groups (placebo non-irradiated, saline irradiated and non-irradiated, and Tinosorb S irradiated and non-irradiated) were accurate representations of the data in this appendix (individual scores). This appeared to be a transcription error, with the correct data showing that the placebo was slightly more reactive than originally indicated in the summary table. Corrected tabulated data showed that the lotion containing Tinosorb S was the least reactive of the three test materials at both non-irradiated and irradiated sites. The difference between the three materials was prominent at irradiated sites, which was probably due to the UV protection provided by residual amounts of Tinosorb S. The only reaction reported was a minimal to slight occurrence of erythema, which was more evident at the saline and placebo treated sites.

However, this error in transcription did not change the overall interpretation of the result, which indicated no effect of Tinosorb S above that of the placebo or saline controls and there was no evidence of a phototoxic response.

> Evaluation of photoallergy by repeated insult patch test in humans (Hill Top Research Inc; Project no. 100243B; J. Plautz; 1998; GCP/QA-yes).

In this study, a total of 33 volunteers (37 screened, 4 failed based on selection criteria) were assessed for a possible photoallergic reaction after repetitive applications of Tinosorb S and exposure to UV radiation. The study included assessment of the effect of control substances (saline and placebo lotion) and Tinosorb S (10%) in a lotion base. The design of the study was a variation on the Kaidbey and Kligman* method and involved sensitising step (maximisation test).

Test materials use in this study were saline (code C), placebo lotion (code D - 779058) and Tinosorb S (10% in placebo lotion; code E - 779057); these test materials were supplied by the sponsor (Ciba Specialty Chemicals) for the study.

Irradiation was based on the MED (minimum erythema dose), which was determined for each subject prior to exposure. Doses of UVA (16 J/cm^2) and UVB (0.75 J/cm^2) were those found to be suitable for both induction and challenge irradiation.

The induction period consisted of a total of 6 applications of each of the materials (saline, lotion placebo and Tinosorb S in lotion) to be tested on the skin of volunteers delivered over a 3 weeks period (2 applications/week). The test materials (0.2 mL neat) were applied to the skin using a Hill Top chamber. The chamber was placed in position on the test sites, which were situated on the paraspinal region of the back. Contact time was 24 hours for the test materials with the skin. Within 10 minutes of removal of the test patches the application sites were exposed to UV radiation (UVA & UVB).

A rest period of 2 weeks, where there was no application of the test materials or exposure to UV radiation, followed the completion of the induction phase.

At challenge, a single application of duplicate patches of each test material was made to naïve sites. The duplicate sites were used to assess either photosensitisation (exposed to UV radiation) or induced contact sensitisation (not exposed to UV radiation). If required, a rechallenge was included in the protocol to reassess subjects for possible false positive or negative reactions. The rechallenge followed the same procedure as the initial challenge. All test sites were evaluated at approximately 1, 24, 48 and 72 hours after UV irradiation and patch removal.

A subject reported sick (adverse event) and was found to have moderate bronchitis, which was considered not to be associated with exposure to the test materials used in this study.

Tabulated data showed that the lotion containing Tinosorb S was the least reactive of the three test materials at both non-irradiated and irradiated sites. The difference between the three materials was prominent at irradiated sites, which was probably due to the UV protection provided by residual amounts of Tinosorb S. The only reaction reported was a minimal to slight occurrence of erythema, which was more evident at the saline and placebo treated sites.

The tabulated data indicated that erythema scores (pink to red) were common place in all groups including saline (unlikely to irritant effect), which maybe related to the erythemic effect of the UV radiation delivered after each application of each test material. This proposal is supported by the data for non-irradiated and irradiated sites at challenge. Non-irradiated sites had significantly less (2-3 fold) observable skin reactivity than irradiated sites. The lotion containing Tinosorb S caused significantly less skin reactivity at both irradiated and non-irradiated sites (compared with saline and placebo lotion) indicating that Tinosorb S (at 10%) was not skin sensitiser or a photoallergen in this study.

Overall, interpretation of the results indicated no effect of Tinosorb S (actually much less reactive than saline or placebo) above that of the placebo or saline controls and there was no evidence of a photoallergy response.

* Kaidbey and Kligman, Photomaximisation test for identifying photoallergen contact sensitisers, Contact Dermatitis, 6, 161-169.

Animal studies

Kinetic data

Metabolic fate following oral administration in rats – Tinosorb S (Central Toxicology Lab. UK; report no CTL/UR0698/REG/REPT; R. Silcock; 2002; GLP/QA-yes).

The excretion of radioactivity in urine and faeces was following in rats (Alpk:Ap$_f$Sd Wistar; 4/sex) given a single oral dose of 50 mg/kg labelled Tinosorb S. Collection of samples took place at regular intervals over a period of 4 days. In a second group (9/sex) of rats, a single dose of 50 mg/kg radiolabelled (stock purity 98.4%) Tinosorb S was followed by blood sampling to determine the time course of absorption of Tinosorb S over a period of 24 hours. It was stated that the dose of 50 mg/kg Tinosorb S represented a non-toxic dose, which was sufficient to follow the metabolic fate of the test substance and allow characterisation of possible metabolites. The dose was delivered by oral gavage in a volume of 4 mL/kg, with the test material suspended in polyethylene glycol 400. Serial blood collection was achieved using the tail vein and terminal blood samples by cardiac puncture. Tissue collected for analysis included reproductive tissues (ovaries, testes, uterus, epididymis and mammary tissue). Data generated was based on results from 3-4 animals per time point.

Excretion was rapid and extensive in the rat (both sexes) following a single oral dose of 50 mg/kg Tinosorb S. Data showed that Tinosorb S was almost entirely excreted in the faeces with 94% and 97% in males and females, respectively. Furthermore, very small amounts of the radiolabel estimated as 0.1% and 0.2% in males and females, respectively, were found in the urine. Analysis of the faeces revealed that unchanged Tinosorb S accounted for the excreted label in both males and females. It was suggested that a proportion of the systemic radioactivity was attributable to radioactive impurities, with the purity of the labelled Tinosorb S in the dose preparation at 97.8%. This proposal was based on blood kinetics seen to be consistent over time, which should have lead to more evidence of uptake despite the very low absorption via the oral route.

Analysis of individual tissue distribution of the label indicated that residues in tissue were extremely low and represented <0.01% of the total dose administered to both males and females. The radioactivity remaining in the carcass was found to be 0.3% in males and 0.1% in females, while total recovery of radioactivity was 95% and 97% for males and females, respectively. Data for reproductive tissues (ovaries, testes, uterus, epididymis and mammary tissue) showed that levels of labelled test material for all these tissues were below the limit of detection.

The concentration of radioactivity in blood and plasma was below the level of detection (blood <0.038 μg/g, plasma <0.019 μg/g) at all analysis points over the 24 hour sampling period. The calculation of C_{max}, $T_{1/2}$ and AUC values was not possible since all blood and plasma levels were below the limit of detection.

It was apparent that there was no significant quantitative difference in the way the test material behaved in either sex. The unchanged parent compound was the only material found in waste products (faeces).

This study indicated that Tinosorb S is poorly absorbed from the GIT (<1%). This information identifies that the oral toxicity studies conducted in support of Tinosorb S would have experienced limited systemic exposure over the course of studies.

Metabolic fate following *in vivo* dermal administration in rats – Tinosorb S (Central Toxicology Lab. UK; report no CTL/UR0722/REG/REPT; R. Silcock; 2002; GLP/QA-yes).

The dermal absorption of radiolabelled 4% Tinosorb S in formulation was investigated for varying dose volumes in the male Alpk:Ap$_f$Sd Wistar rat. Volumes used were 22 and 54 µL of the formulated dose, which were applied to 10cm^2 of clipped skin/rat; these volumes equated to doses of 2.0 and 0.8 mg Tinosorb S/rat. It was noted that the dose applied approximated likely human dermal exposure to a sunscreen formulation during normal use. It was noted that the strain of rat used in this study was the same as used in toxicity studies presented in this submission.

The study protocol involved 32 rats each given a single dermal dose of either 2 mg or 0.8 mg labelled Tinosorb S. After application of the test material, the treated sites were protected using O-rings (non-occlusive) incorporating a nylon gauze cover. The O-rings were kept in place with a non-occlusive elasticised bandage. Animals were individually housed in metabolism cages for collection of urine and faeces. Exposure time to dermally applied Tinosorb S was 6 hours, after which time the O-ring was removed and the skin was washed to remove unabsorbed residues. Groups of 4 rats were terminated at 6, 24, 72 and 120 hours after dosing to enable sample collection and analysis of relevant kinetic parameters. The skin under the protective O-rings was washed (using soap solution in water) to remove unabsorbed residue and the application sites were then tape-stripped to remove the stratum corneum. All samples, including selected tissues and residual carcass were analysed for radioactivity. Analysis for each time point and dose was based on data from 4 animals.

In animals exposed to 2 mg Tinosorb S, the amount absorbed after 6 and 24 hours was 0.2% of the applied dose. Approximately 90% of the applied radioactivity had been washed from the skin at the end of the exposure period (6 hours). Approximately 2.0% of the dose had been estimated to be associated with the structures (skin layers) at the treatment sites, with an undetermined amount available for absorption. The residual radioactivity at the treatment sites did decline slightly in amount at the later evaluation times.

At the lower dose of 0.8 mg, the amount absorbed after 6 and 24 hours was 0.1-0.4% of the applied dose. Approximately 96% of the applied radioactivity had been washed from the skin at the end of the exposure period (6 hours). Approximately 2.1% of the dose had been estimated to be associated with the structures (skin layers) at the treatment sites, with an undetermined amount available for absorption. The residual radioactivity at the treatment sites did decline slightly in amount at the later evaluation times.

This study indicated that Tinosorb S is poorly absorbed through the skin (<1%). Toxicity studies conducted using dermal administration of Tinosorb S would have experienced limited systemic exposure. Information from this and the preceding study identifies that absorption (systemic exposure) of Tinosorb S is of a similar magnitude following its oral or dermal administration in the rat.

Local Tolerance Studies

Local Irritation

> Primary skin irritation study in the rabbit (Research & Consulting Company [RCC] Ltd.; report no. 651431; W. Braun; 1997; GLP/QA-yes).

In this study, Tinosorb S was examined for skin irritation potential following topical application of 0.5 g to an area of 6 cm^2 of intact skin (clipped) on the dorsal surface of 3 NZ rabbits (1 male, 2 females). The test material was moistened in distilled water prior to application, which was for a 4 hours contact period. The test material was covered with a semi-occlusive dressing to prevent loss. Parameters examined included erythema/eschar, oedema, lesions and corrosiveness. The treated skin was assessed and graded for skin reactions at 1, 24, 48 and 72 hours after removal of the dressing. An index of primary irritation was calculated from the results at each assessment time point.

Animals were symptom free, with no overt clinical signs of toxicity detected for the duration of the study. The estimated value of primary irritation was zero based on a lack of skin reaction at all time points. There was no evidence of erythema or oedema on the treated surface. It was noted that the test material caused reversible staining of a light yellow colour of the treated skin. Examination of the treated skin failed to detect any evidence of local corrosive activity at any of the assessment time points. Based on established criteria for skin reactivity (EEC Directive 93/21/EEC), the test material was not a skin irritant under the conditions described above.

> Primary eye irritation study in the rabbit (Research & Consulting Company [RCC] Ltd.; report no. 651442; W. Braun; 1997; GLP/QA-yes).

In this study, Tinosorb S was examined for eye irritation potential following instillation of 0.1 g into one eye of each of 3 (1 male, 2 females) NZ rabbits. The treated eye was not rinsed following instillation of the test material into the eye. Assessment of the treated eye took place 1, 24, 48 and 72 hours after initial application of the test material. A primary ocular irritation index was estimated from the reaction scores at each of the time points.

There were no clinical signs of systemic toxicity observed during the study period and there were no deaths. The body weight of the study animals was within the normal range for this species and strain.

The primary irritation score, calculated from the individual cumulative scores, resulted in a value of 0.44 (max. 13) determined for the primary irritation index. The index of 0.44 was based on slight reddening and swelling of the conjunctivae, which had resolved by 48 hours after exposure to the test material. Examination of the eye revealed no staining of the cornea, sclera or conjunctivae in the eye treated with the test material. There was no evidence of corrosion of any of the structures within the eye. Based on these findings the test material was found to be non-irritating to the eye of the rabbit.

Local tolerance study after repeated topical application for 2 weeks in guinea pigs (CIT Labs, France; study no. 23972TSG; B. Griffon; 2002; GLP/QA-yes).

This study involved the repeated cutaneous application of the test material (Tinosorb S) for 2 weeks at concentrations of 3% and 10% to the skin of guinea pigs. A total of 15 male Hartley Crl(HA)BR guinea pigs divided into 3 groups of 5 animals each. The groups were treated with 10% test material, 3% test material or vehicle (diethoxyethyl succinate) on the right clipped flank, while the left clipped flank remained as an untreated control. Each animal had its assigned treatment once daily (same time each day), five days a week for a 2 weeks period. A volume of 0.3 mL of each treatment was used and dressings were not placed over the test sites. Application sites were clipped free of fur on days1, 4, 8 and 11 at least 3 hours before treatment, as well as 3 hours before scoring on day 15. Cutaneous reactions were assessed before each application and on day 15. Cutaneous reactions such as erythema, oedema and desquamation were used to estimate a cumulative irritation index.

There were no clinical signs of toxicity (observation made daily) or deaths during the course of the study. In the group receiving 10% test material, a slight erythema was observed in all animals on days 4 and 8, in 4/5 animals on day 5 and in 1/5 animal on days 9, 10, 11 and 15. A cumulative irritation index of 1.0 was determined.

In the group receiving 3% test material, a slight erythema and desquamation were observed in a few animals on days 4 through to 11, with nothing after this time. A cumulative irritation index of 0.3 was determined. It was noted that the control flank did display slight erythema in 3/5 animals and slight desquamation in 1/5 animals on day 8. These findings were equivalent to an index rating of 0.1.

In the vehicle control group, slight erythema was observed in 2/5 animals on day 8. These responses were described as slight and were suggested to be a consequence of clipping the fur from the skin. A cumulative index score of zero was assigned to this group.

It was concluded that percutaneous application of Tinosorb S to the shaved skin of guinea pigs resulted in slight irritation, which was apparently dose-dependent based on irritation index scores of 1.0, 0.3 and 0.0 for concentrations of 10%, 35 and zero (vehicle). The scoring system was based on a scale of 0, 1, 2 or 3 for severity of reaction. A score greater than (2 – moderate on day 8 only) 1 was only seen in one animal at one time point in the group receiving 10% Tinosorb S. All other observed reactions were rated as being of slight intensity (1 on scale) for erythema, oedema or desquamation.

Contact hypersensitivity to Tinosorb S in albino guinea pigs – maximisation test (RCC Lab; report no. 651453; G. Arcelin; 1997; GLP/QA-yes).

A skin sensitisation test (maximisation assay) was conducted using male Himalayan spotted guinea pigs (15 total, 10 test and 5 control). The induction protocol consisted of a 3% dilution of the test material in PEG400 and in an emulsion with Freund’s Complete Adjunct in physiological saline, which was delivered intra-dermally (0.1 mL). Sodium lauryl sulfate (SLS) was massaged into the application sites to provoke a slight inflammatory response that enhances sensitisation.

There were 2 treatments by intra-dermal injection, which were spaced 1 week apart. One week after the last intra-dermal injection patches saturated (approx. 0.3 g) with 30% test material were applied to the flanks of the animals and left in place for 24 hours. The epicutaneous induction part of the protocol was carried out with 30% test material in PEG400, which was covered by an occlusive dressing. The challenge was initiated 2 weeks after the last induction dose and consisted of an epicutaneous dose of 30% test material in PEG400 covered by an occlusive dressing. A vehicle control group was included in the study. Skin reactions to the test material applied during the induction procedure were monitored and the challenge response was evaluated at 24 and 48 hours after removal of the dressing from the challenge site. The concentrations used in this study were determined following a dose-range finding assessment.

There were no deaths in either the test or control groups during the course of the study. Animals did not display any clinical signs of toxicity and their body weights were within the normal range for animals of this species and strain.

Similar local skin responses (described as normal) were observed in both test animals and controls following intra-dermal induction. Assessment of skin reactivity during the induction procedure found a similar intensity (slight erythema) and frequency (40%) of reaction in both test animals and controls. It was suggested that application of SLS was probably responsible for the slight erythema seen in both test and control animals at the same frequency. At challenge, there was evidence of skin reactions to the test material or vehicle at either assessment time (24 & 48 hours). It was concluded that the test material was not a skin sensitiser in the guinea pig under conditions of heightened responsiveness (maximisation criteria).

> Determination of phototoxicity with Tinosorb S in albino guinea pigs (RCC Lab; report no. 651475; G. Arcelin; 1997; GLP/QA-yes).

The phototoxicity of Tinosorb S was investigated in female albino Dunkin Hartley guinea pigs. This study included a test group (10 animals) that had Tinosorb S (concentrations of 10%, 15%, 25% and 30%) applied dermally to the shaven skin (left flank) on areas of approximately 2 cm^2 prior to irradiation with 20 J/cm^2 UVA (over 320-400 nm). The test material was applied with a spatula to the prepared skin on the left flank in order to saturate these sites. Irradiation took place 30 minutes after application of the test material. The right flank was not exposed to the light and was used as a reference site. The test material was dissolved in polyethylene glycol 400 (PEG400). A control group (5 animals) was exposed to UV radiation in a similar manner to the test group, but controls had only the vehicle (PEG400) applied to the irradiated sites. Reactions of the skin (erythema/oedema) in both treated and controls were assessed at 24, 48 and 72 hours after application. It was noted that all animals were pretreated with 2% DMSO in ethanol to enhance the skin penetration of the test material.

There were no deaths during the course of the study. All animals were free of either local or systemic signs of toxicity over the course of the study. Body weights of all animals remained within the normal range for this strain of guinea pig. There were no reactions on the right flank, which had been used as a non-irradiated reference site. The test group reaction profile was 0/10 skin reactions at every concentration used and at every time point of assessment. Similarly, the

controls also had zero reactions at all time points of assessment. Therefore, it was concluded that the test material did not induce phototoxicity under the test conditions described above.

> Determination of photoallergenicity with Tinosorb S in albino guinea pigs (RCC Lab; report no. 651497; G. Arcelin; 1997; GLP/QA-yes).

The primary focus of this study was to investigate the potential photoallergenicity of Tinosorb S, but it was noted that information about allergenicity, photoirritation and irritation would be described. In accordance with guidelines, a total of 30 guinea pigs (Dunkin Hartley strain) were used with 20 in the test group and 10 controls. Tinosorb S was prepared for use by dissolving it in polyethylene glycol 400 prior to the application of a 30% concentration (0.1 mL) to the skin (8 cm^2). Levels of Tinosorb S used were based on the highest non-irritating concentration of 30%. The induction phase also included a priming process involving 4 intradermal injections (0.1 mL) of Freund's Complete Adjuvant (FCA) at each of the test sites. After this initial treatment the test sites were exposed to 1.8 J/cm^2 UVB and 10 J/cm^2 UVA irradiation. This format of test material application and UV irradiation was repeated 4 times within the 2 weeks induction phase. Control animals were treated with FCA and underwent the process described for induction minus the test material.

Challenge was initiated 3 weeks after the start of the induction phase and involved epicutaneous treatment (0.025 mL/cm^2) of the study animals on both flanks with the test material at concentrations of 10%, 15%, 25% and 30%. Treated sites were then either exposed to UV irradiation (left flank) or left unirradiated (right flank). Reactions of the skin (erythema, oedema etc) were evaluated at 24, 48 and 72 hours after the challenge.

There were no deaths during the course of the study. There were no clinical signs of toxicity observed over the study and body weights were within the normal range for guinea pigs of this strain.

Intradermal injection of FCA generated findings described as normal for this procedure, which were characterised by erythema, oedema, necrotising dermatitis and exfoliation of encrustation. It was noted that skin effects associated with Tinosorb S during induction were slight erythema/oedema, which were linked to the concurrent use of FCA. A satellite positive control group was included using 3,3',4'5-tetrachlorosalicylanilide in the presence and absence of UV irradiation. This agent generated a significant frequency and severity of response to validate the methodology.

Tabulated data for the challenge period in treated animals and controls showed that no reactions were recorded for any test material concentration at any of the assessment time points. All concentrations and time points for test and control animals under irradiated and non-irradiated conditions were negative for skin reactions. Overall, it was evident that Tinosorb S did not induce a photoallergenic response in the guinea pig under maximisation (use of FCA) conditions.

Toxicity Studies

Acute toxicity

Acute dermal toxicity study with Tinosorb S in the rat (RCC Lab; report no. 651420; G. Arcelin; 1997; GLP/QA-yes).

A group (5/sex) of HanIbm:WIST(SPF) rats had Tinosorb S applied to clipped fur at 2000 mg/kg. The test article was suspended in PEG400 at a concentration of 0.5 g/mL and administered at a volume of 4 mL/kg. The animals were monitored for clinical signs of toxicity at regular intervals (4 times) on day 1 and once daily from day 2 to 15. Survival, body weight and necropsy were examined in the course of the study.

There were no clinical signs of toxicity or deaths during the course of the study. Examination of the application sites revealed no adverse effects of treatment on the skin. The body weights of animals remained within the normal range for animals of this species and strain. At necropsy, there was no evidence of treatment-related effects on organs/tissues examination at termination of the study. A LD50 value of >2 g/kg was estimated for the dermal application of Tinosorb S.

Acute oral toxicity study with Tinosorb S in rats (RCC Lab; report no. 651407; G. Arcelin; 1997; GLP/QA-yes).

A group (5/sex) of HanIbm:Wistar (SPF) rats had Tinosorb S administered at 2000 mg/kg by oral gavage. Tinosorb S was suspended in polyethylene glycol 400 (vehicle) at a concentration of 0.2 g/mL and administered in a volume of 10 mL/kg body weight. The animals were monitored for clinical signs of toxicity 4 times on day 1 and then daily over the 15 days observation period. Survival and viability were monitored over the same time frame. Body weights of animals were measured on day 1, and again on days 8 and 15 of the study. At the end of the monitoring period all animals were sacrificed and examined macroscopically.

There were no deaths recorded or clinical signs of toxicity observed during the course of the study. Body weights of study animals were within the normal range for this strain of rat and the findings at necropsy were unremarkable. A LD50 value of > 2 g/kg was estimated in the rat for orally administered Tinosorb S.

Sub-chronic dermal toxicity

Preliminary 2 weeks toxicity study by cutaneous route in rats (CIT Labs, France; report no. 25455TSR; I. Gaou; 2003; QA-yes).

This study acted as a dose-range finding investigation for a main study of 13 weeks duration. Two groups (5/sex) of Wistar Han rats had Tinosorb S (1000 mg/kg/day) or vehicle (PEG 400) applied daily to their clipped skin for a period of 2 weeks. The animals were monitored daily for clinical signs of toxicity and survival. Body weight and food consumption changes were measured twice a week during the course of the study. An autopsy was conducted on at the end of the treatment period.

There were no deaths during the course of the study. There were no clinical signs of systemic toxicity, while limited skin reactions (scabs on 1 treated female; slight desquamation on 1 control

female and the majority of treated females) were observed on treated and controls. There were no differences between treated animals and controls in the areas of body weight gain or food consumption. Autopsies on the study animals at the end of the study were unremarkable. Overall, it was decided that the daily dermal application of 1000 mg/kg Tinosorb S was well tolerated by rats and this dose was suitable for a longer 13 weeks study.

A 13 weeks toxicity study by cutaneous route in rats (CIT Labs, France; report no. 25378TCR; I. Gaou; 2003; GLP/QA-yes).

In this study, groups (13/sex/dose) of Wistar Han rats had Tinosorb S (0, 250, 500 or 1000 mg/kg/day) applied to their clipped skin daily for a period of 13 weeks. Included in this study were satellite groups for the assessment of the effect of a protective collar (only at 1000 mg/kg/day dose) and kinetic parameters (plasma drug levels) at various time points (day 8, weeks 6 and 13). Polyethylene glycol 400 was the vehicle used in this study. Animals were examined on a daily basis for survival and clinical signs of toxicity. A weekly detailed clinical examination was also conducted. Other parameters monitored on a weekly basis were body weight and food consumption. Ophthalmological examinations were performed on vehicle and high dose animals. In females, the oestrous cycle was examined to determine if treatment modified normal function. Haematology, clinical chemistry and urinalysis were all examined at the end of the treatment period. Both macroscopic and microscopic examinations of study animals were conducted at the end of the study. An additional assessment involved a battery of neurotoxic assays used to determine whether Tinosorb S had any adverse effects on a number of developmental parameters.

There were no deaths or clinical signs of systemic or cutaneous toxicity during the course of the study. Examination of the skin for reaction to the test material looked for desquamation, scabs, erythema and cutaneous lesions. A control female was the only animal found to have a defined cutaneous lesion. There were no observable incidences of desquamation, scabs or erythema in controls. Desquamation was only observed in treated males and not in a dose-related manner. It was noted that residues of the test material on the application site resembled desquamation, which may explain the low incidence in males only. Scabs and erythema were seen in both males in females at a low incidence and not in a dose–related manner. These findings were limited to the first 10 days of dosing and were no longer present after this time. Animals with collars on to limit licking showed no evidence of erythema and a very low (compared to non-collar animals) incidence of desquamation and scabs. It was considered that the skin reactions seen in treated animals were unlikely to be directly related to treatment with Tinosorb S, but more likely to be due to the presence of residue at the application site or licking (causing slight irritation) by the animals.

Data on body weight gain (± 5% from vehicle control, not dose-related) and food consumption failed to show any difference between the treatment groups and controls. Ophthalmological examination was unremarkable and the oestrus cycle was unaffected by treatment with Tinosorb S.

Clinical chemistry, haematology, urinalysis and neurological examination were unremarkable, with no evidence of any treatment-related adverse effects. Slight fluctuations in mean cell volume and mean haemoglobin concentration were noted, but these were not dose-related, were seen in one sex and not the other, and were within the normal physiological range and seen in the absence of relevant histopathological changes. These effects were considered not to be treatment-related. The were similar findings for a number of clinical chemistry parameters (sodium, potassium, calcium, albumin, triglyceride levels and hepatic enzyme activities), which were considered not to be treatment-related.

Data on the plasma levels of Tinosorb S revealed very low dermal absorption and systemic exposure, which suggested that it was not bioavailable from the cutaneous route of administration. The limit of quantification was 2 ng/mL. Plasma levels in control animals were below the limit of detection. In uncollared animals receiving 1000 mg/kg/day, the amount of test material at day 8 was below the limit of detection. At day 37 it was 7.53 ng/mL in males and not detected in females and at day 91 levels of 4.81 and 6.51 ng/ml were measured for males and females, respectively. In collared animals receiving 1000 mg/kg/day, the amount of test material at day 8 was below the limit of detection. At day 37 it was 10.00 ng/mL in males and 6.35 ng/mL in females and at day 91 levels were below the limit of detection for both males and females.

Organ weight analysis, macroscopic and microscopic findings were all unremarkable, with no evidence of any adverse effects associated with dermal exposure to Tinosorb S. Microscopic examination of treatment sites for test animals and controls showed minimal to slight hyperkeratosis sometimes with parakeratosis, acanthosis, spongiosis and empty hair follicles. The incidence and severity of these findings were not dose-related and without prominent differences from the controls (negative and vehicle sites). It was suggested that these changes were due to mechanical injuries incurred during dose-site preparation (clipping and cleaning) and were not due to an effect of the test material.

Overall, the NEL for toxicity in this study was estimated to be 1000 mg/kg/day in rats administered Tinosorb S by the cutaneous route.

> 14 days range-finding study with orally administered Tinosorb S in the rat (RCC Lab; report no. 667530; H. Schmid; 1998; QA-yes).

The aim of this study was establish suitable dose levels to be used in a longer-term oral toxicity study. Groups of (5/sex) of HanIbm:Wistar (SPF) rats had Tinosorb S administered at 0, 50, 200, 800 and 2000 mg/kg/day by oral gavage over a period of 2 weeks. Tinosorb S was suspended in polyethylene glycol 400 (vehicle) and administered in a volume of 10 mL/kg body weight. Clinical signs of toxicity were assessed twice daily. Food consumption was recorded on a weekly basis, while body weights were assessed twice a week. Ophthalmoscopic examination was conducted on all animals. Clinical chemistry and haematological analysis was conducted on samples taken after the 14 days treatment period. Necropsies were conducted on all animals at the end of the treatment period, as well as organ weights. Histopathological examination was conducted on tissues from the high dose group and controls.

There were no deaths in any group during the study. Clinical signs of toxicity were absence in the groups receiving 50, 200 and 800 mg/kg/day, while the only treatment-related finding was the occurrence of pale faeces in all rats receiving 2000 mg/kg/day. This finding was related to the considerable amount of test material ingested and its yellow colour. Pale faeces did not appear to be of toxicological relevance, since there were no other adverse effects that could associated with GIT abnormalities.

Food consumption and body weight development were consistent across all groups and fell with expected (historical data for rat) limits over the course of the study. Haematology, clinical

chemistry and ophthalmological data were unremarkable, with no overt variations across all groups. Organ weight analysis did not reveal any evidence of treatment-related effects. Examination of animals both macroscopically and microscopically identified an array of spontaneous tissue aberrations that could not be associated with exposure to the test material.

These results were used to suggest appropriate dose levels of 50, 250 or 1000 mg/kg/day for a longer-term (13 weeks) study to be conducted in rats. However, the protocol for the 13 weeks study states that dose levels of 100, 500 or 1000 mg/kg/day were used.

> Thirteen-week oral toxicity study with Tinosorb S in the rat (RCC Lab; report no. 667541; H-J. Hamann; 1998; GLP/QA-yes).

In this 13 weeks oral toxicity study, groups of (20/sex) of HanIbm:Wistar (SPF) rats had Tinosorb S administered at 0, 100, 500 and 1000 mg/kg/day by oral gavage. Tinosorb S (yellow solid) was suspended in polyethylene glycol 400 (vehicle) and administered in a volume of 5 mL/kg body weight. Clinical signs of toxicity were assessed at least twice daily and viability/mortality on a twice daily basis. Food consumption and body weights were recorded on a weekly basis. Ophthalmoscopic examination was conducted on all animals at the end of treatment. Included in the health assessment of animals was a functional observational battery of tests conducted at pre-test, and weeks 6 and 12 of the study. These tests included locomotor activity, startle response, alertness, aberrant behaviour, pilo-erection, tremor, twitches, spasm, body carriage and gait, exploratory activity and sedation. Further monitoring included parameters that would be picked up in the daily examination for clinical signs of toxicity.

At 5, 9 and 13 weeks blood samples were taken to enable clinical chemistry and haematological analysis to be conducted. In addition, urine samples (for analysis) were collected at these times using metabolism cages. Necropsies were conducted on all animals at the end of the 13 weeks treatment period, including the measurement of organ weights (for adrenals, brain, kidneys, liver, spleen, testes, thyroid and thymus). Histopathological examination was conducted on tissues from all treatment groups and controls.

There were no treatment-related deaths during the study and clinical signs of toxicity were absence in all groups (0, 100, 500 and 1000 mg/kg/day). A total of 3 male rats (1 control, 2 LD) died spontaneously between days 42 and 81 of the study. There were 2 females (1 control, 1 HD) that died spontaneously day 91. An autopsy revealed that all 5 animals (3 males, 2 females) had died due to accidental administration of the test material into their airway. A further 2 rats (1 male, 1 female) died accidentally as a result of the blood sampling procedure. Clinical signs observed during the course of the study included dyspnea, alopecia, reddish sores around neck and necrosis (crusting) of cervical region and on left shoulder. These findings occurred in a random manner across all groups.

The functional observational battery of tests described above were unaffected by treatment with Tinosorb S at all dose levels. Incidental findings included slightly decreased exploratory activity, slight vocalisation and slight fearfulness, which occurred in a limit umber of animals. Observed fore-limb and hind-limb grip strength was increased in males and decreased in females at

different stages in the study; this effect lacked a dose-response relationship and went in opposite directions in the different sexes.

Body weight gain and food consumption were consistent in all treatment groups and controls, indicating no adverse effect associated with exposure to Tinosorb S up to 1000 mg/kg/day PO. The variation in body weight gain between groups was less than 5%.

Ophthalmological examination was unremarkable, with no differences in the state/function of eyes in treatment groups and controls. Findings in a limit number of animals from all groups included persistent membranes (1 control, 1 LD, 2 MD, 0 HD), non-responsiveness to mydriatic agent (1 HD) and haemorrhagia in the vitreous body (1 control).

Haematological parameters measured at 5, 9 and 13 weeks were unaffected following exposure to Tinosorb S up to 1000 mg/kg/day. Particular mention was made of possible cellular markers (total leucocyte count, lymphocytes, monocytes and polymorphonuclear leucocytes) of immune function. In the data presented there was only one instance where a value (differential count – segmented neutrophil) was seen as significantly difference from the control value and this occurred only in LD males. It was stated that there were no treatment-related changes in these cell populations, which suggests that there was no adverse effect on the immune system.

Data showed that there were no changes in clinical chemistry and urinalysis parameters of toxicological significance at any of the sampling times. The gamma globin fraction in the protein electrophoretic pattern was slightly reduced in HD males at weeks 5 and 9, and in both HD sexes at week 13. Parameters (protein [differential components], cholesterol, triglycerides, liver enzymes) indicative of liver damage/activity were unaffected by treatment with any slight variation in these values found to be within the normal range and lacking dose relationships.

Urine output was slightly, but non-significantly increased in MD and HD females at weeks 5 and 9, and in both sexes at the HD at week 13. The urine output in all treated males was lower than the controls at weeks 5 and 9. A comparison of control data over the course of the study revealed a reduction (up to 36%) in urine output in both sexes at week 13 compared to the earlier assessment times. It was noted that observed inter-group differences were slight and not dose-related, often only occurred in one sex and any fluctuations were within the historical background range for this strain of rat. Furthermore, these effects occurred in isolation from any relevant changes in structure or function in associated tissues/organs. A significant decrease in specific gravity was seen at the HD, but this fluctuation was less than 2% of the control value.

Organ weights (relative and absolute) were similar across all groups, indicating Tinosorb S had no adverse effects up to 1000 mg/kg/day PO. Virtually no organ weight (relative or absolute) was found to be significantly different from its corresponding control. A finding of decreased (12%) mean absolute liver weight in LD females at autopsy was considered to be an isolated (not treatment-related) occurrence since there was no dose-relationship, it was only detected in one sex and it did not correlate with histopathological findings.

The results from the necropsy were unremarkable, with no evidence of treatment-related adverse effects. Histopathology was also unremarkable. The incidence of macroscopic and microscopic aberrations was limited and occurred at a similar frequency across all study groups.

Overall, the oral administration of Tinosorb S at doses up to 1000 mg/kg/day for 13 weeks in rats did not lead to overt toxicity. A NEL for this study was estimated at 1000 mg/kg/day Tinosorb S.

Genotoxicity Studies

Escherichia coli reverse mutation assay (RCC Lab; report no. 618300; H-E. Wollny; 1998; GLP/QA-yes).

Tinosorb S was tested for its ability to induce gene mutations in E. coli (strain WP2uvrA) according to plate incorporation and pre-incubation methodology. The assay was performed in 2 independent experiments both in the presence and absence of metabolic activation (rat liver S9 fraction). Concentrations of the test material were assayed in triplicate and the assays included vehicle (DMSO) and positive (methyl methane sulfonate; 2-aminoanthracene) controls. Pre-testing for an appropriate concentration of test material showed normal culture growth up to 5000 μg/plate in the presence and absence of metabolic activation. Concentrations of the test material used in this study were 0, 33, 100, 333, 1000, 2500 and 5000 μg/plate.

There was no evidence of significant cell toxicity at any concentration in the presence or absence of metabolic activation. There was no evidence of a significant increase in revertant colony numbers at any concentration of the test material in the presence or absence of metabolic activation. Revertant numbers/plate across all concentration of the test material did not exceed a ratio of 1.6 (compared to vehicle control, range 0.9-1.6) and there was no suggestion of a concentration-related increase. Positive controls generated an anticipated increase in revertant colony numbers to validate the assay procedure.

Overall, the test material did not induce gene mutations (base-pair changes) in cultures of E. coli (strain WP2uvrA) cells at the concentrations tested in this assay.

Salmonella typhimurium reverse mutation assay (RCC Lab; report no. 651508; H-E. Wollny; 1997; GLP/QA-yes).

Tinosorb S was tested for its ability to induce gene mutations in Salmonella typhimurium (strains TA1535, TA1537, TA98 and TA100) according to plate incorporation and pre-incubation methodology. The assay was performed in 2 independent experiments both in the presence and absence of metabolic activation (rat liver S9 fraction). Concentrations of the test material were assayed in triplicate and the assays included vehicle (DMSO) and positive (sodium azide; 4-nitro-o-phenylene-diamine; 2-aminoanthracene) controls. Pre-testing for an appropriate concentration of test material in the presence and absence of metabolic activation was conducted to establish the concentrations used in the assay. Concentrations of the test material used in this study were 0, 33.3, 100.0, 333.3, 1000.0, 2500.0 and 5000.0 μg/plate.

There was evidence of slight toxicity at higher concentrations (≥2500.0) in the presence or absence of metabolic activation. There was no evidence of a significant increase in revertant colony numbers at any concentration of the test material in the presence or absence of metabolic activation. Revertant numbers/plate across all concentrations of the test material and for all strains did not exceed a ratio of 1.7 (compared to vehicle control; range 0.5-1.7) and there was no suggestion of a concentration-related increase (greatest ratio of 1.7 seen at lowest concentration). Positive controls generated an anticipated increase in revertant colony numbers to validate the assay procedure.

Overall, the test material did not induce gene mutations (frame-shift/base-pair changes) in cultures of Salmonella typhimurium (strains TA1535, TA1537, TA98 and TA100) cells at the concentrations tested in this assay.

Photo-mutagenicity in a Salmonella typhimurium and E. coli reverse mutation assay (RCC Lab; report no. 609003; H-E. Wollny; 1998; GLP/QA-yes).

Tinosorb S was tested for its ability to induce gene mutations (reverse mutation) in Salmonella typhimurium (strain TA102) and Escherichia coli (strain WP2uvrA) under conditions of irradiation with artificial sunlight. The procedures were similar to those described in the previous two studies with Salmonella typhimurium and Escherichia coli, with concentrations of 0, 33, 100, 333, 1000, 2500 and 5000 μg/plate test material. These strains were selected since they tolerate relatively high exposures of UV radiation used to assess photo-mutagenic potential. Appropriate levels (optimal) of UV exposure were established in a preliminary assay. Levels of UV radiation chosen were 10-40 seconds of 20-80 mJ/cm^2 UVA and 1-4 mJ/cm^2 of UVB; the levels of exposure varied for the different bacterial species and strains. Positive (methyl methane sulfonate), vehicle (DMSO) and negative control groups were included in the study. This study did not incorporate the use of metabolic activation.

Plates incubated with the test material displayed a normal background growth up to the highest concentration of test material. In the preliminary testing assay, irradiation times below 90 seconds did induce a slight increase (<2 fold) in revertant numbers when compared to non-irradiated cultures. Revertant numbers for the TA102 and WP2 strains following exposure to UV radiation were similar to the vehicle and negative control values, with the ratio (revertant test material/revertant control) for both strains in the range of 0.5 to 1.0 for the concentration of test material. The positive control generated appropriate increases in revertant numbers validating the assay procedure.

Overall, the test material did not induce gene mutations (frame-shift/base-pair changes) in cultures of Salmonella typhimurium (TA100) and Escherichia coli (WP2uvrA) cells at the concentrations tested in this assay and following exposure to UV radiation.

In vitro chromosome aberration assay in Chinese hamster V79 cells with Tinosorb S (RCC Lab; report no. 672017; A. Czich; 1998; GLP/QA-yes).

Tinosorb S was examined for its ability to induce structural chromosomal aberrations in cultured V79 Chinese hamster cells. The test material was assessed in the presence and absence of metabolic activation (S9). Concentrations (6.4-210 μg/mL –S9; 3.3-210 μg/mL +S9) of the test material were added to cultures of the cells and 18 and 28 hours after initial incubation began the cells were recovered and examined for the presence of chromosomal aberrations (100 metaphase plates scored for aberrations). Experiments were conducted in duplicate. There was no evidence of cytotoxicity at the concentrations used, but it was indicated that there was some precipitation of the test material. Positive (ethylmethane sulfonate; cyclophosphamide) and vehicle (acetone) controls were included in the study.

The data showed that there was no significant increase in the frequency polyploid metaphases in treatment groups when compared to controls. The frequency of aberrant cells with gaps or exchanges was within normal range. There was an instance where the frequency of gaps was statistically significantly increased (3.5% vs 0.5% control) in cells harvested at 28 hours after exposure to 210 μg/mL in experiment 2. However, this result was influenced by an abnormally low (0.5%) concurrent control value when you consider the majority of other control values for this parameter were 2.5%-3.0%. It was also noted that the historical control value for this parameter has a range of 0.5% to 4.0%, which indicates this significant result was within a normal biological range established for these laboratories. Also, the duplicate assay generated a 0.0% result for gaps under the same conditions for concentration of test material and harvest time. It was justifiably determined that the increased frequency of gaps at 28 hours harvest and 210 μg/mL test material was not a biological significant event. Positive controls induced significant increases in aberrations to support the acceptance of the protocol used in testing.

Overall, Tinosorb S was found not to induce chromosomal aberrations in V79 Chinese hamster cells in the presence or absence of metabolic activation.

> *In vitro* photo-mutagenicity chromosomal aberration assay in Chinese hamster V79 cells with Tinosorb S (RCC Lab; report no. 615904; A. Czich; 1998; GLP/QA-yes).

Tinosorb S was examined for its ability to induce structural chromosomal aberrations in cultured V79 Chinese hamster cells in the presence and absence of UV radiation (UVA 200-300 mJ/cm^2 & UVB 22-33 mJ/cm^2). Concentrations (6.5-100 μg/mL) of the test material were added to cultures of the cells and 18 and 28 hours after initial incubation began the cells were recovered and examined for the presence of chromosomal aberrations (100 metaphase plates scored for aberrations). Experiments were conducted in duplicate. The concentrations of test material were selected based on solubility and cytotoxicity, and the UV radiation dose based on cytotoxicity. Positive (ethylmethane sulfonate; 8-methoxypsoralene), negative and vehicle (acetone) controls were included in the study. The lower concentration range of test material was related to the combined toxicity of the test material and exposure to UV radiation.

The data showed that there was no significant increase in the frequency of polyploid metaphases in treatment groups when compared to controls. The results showed that the test material did not induce an increase in frequency of cells with structural chromosomal aberrations in the presence or absence of metabolic activation. Values for frequency of treated cells with chromosomal aberrations were similar to the concurrent control values in the duplicate experiments. On the odd occasion when the frequency of chromosomal aberration was elevated (non-significantly) there was never any evidence of a concentration-relationship. The values for chromosomal aberration were within the historical value for the laboratory. Positive controls induced significant increases in aberrations to support the acceptance of the protocol used in testing. The following table summarises results for this study.

Frequency of aberrant cell % range

	Incl. gaps	Excl. gaps	Exchanges
Vehicle control	1.5 – 3.5	0.5 – 2.5	0.0 – 0.5
Negative control	1.0 – 4.0	0.5 – 2.5	0.0 – 0.5

Historical control	0.0 – 6.0	0.0 – 6.0	0.0 – 2.0
Positive control	7.5 – 26.5	7.0 – 24.0	2.5 – 9.5
Test material (all doses)	0.5 – 6.0	0.5 – 4.0	0.0 – 1.0

Bone marrow micronucleus test by intraperitoneal route in mice (CIT Labs; report no. 25608MAS; H. Haddouk; 2003; GLP/QA-yes).

The study examined the potential of Tinosorb S to cause an increase in the number of micronucleated polychromatic erythrocytes in the bone marrow of mice. The formation of micronuclei following exposure to a chemical relates to adverse effects directed toward genetic events. A preliminary dose-range finding assessment was conducted to establish appropriate doses for use in the main section of this study.

In the main study, a total of 4 groups (5/sex/dose) of Swiss Ico:OFI (IOPPS Caw) mice were given intraperitoneal administrations of Tinosorb S at dose-levels of 0 (corn oil), 500, 1000 or 2000 mg/kg/day, over a 2 day period. An additional group received a positive control substance (cyclophosphamide 50 mg/kg). Animals from the test and vehicle control groups were sacrificed 24 hours after the last dose, while animals receiving the positive control substance were sacrificed 24 hours after a single dose. The femur was removed from the sacrificed animals and bone marrow smears prepared for analysis. Analysis required determination of micronucleated polychromatic erythrocytes (MPE) to be assessed by counting 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes.
It was noted that the highest dose used was selected based on established criteria that if no toxicity is observed up to 2000 mg/kg then this will become the top dose. Study animals were monitored for signs of toxicity after administration of the test material, but none were observed even at 2000 mg/kg/day and there were no deaths. In this study, the mean values for frequency of MPE and PE/NE were similar in the treatment groups and the vehicle controls. The frequency of MPE/1000PE was ≤ 1 for the test groups and vehicle controls, while the positive control generated mean scores of 23.2 and 27.6 in females and males, respectively. The PE/NE ratio was < 1 for all groups including vehicle and positive controls.

Tinosorb S was considered not to be mutagenic in a mouse micronucleus assay at doses up to 2000 mg/kg/day.

Reproductive toxicity

Dose range-finding prenatal toxicity study with Tinosorb S in the rat (RCC Labs; report no. 681480; H. Becker; 1998; QA-yes).

This study was conducted to establish appropriate dose levels to be used in a main reproductive toxicity study. Groups (5/dose) of mated female Wistar rats had Tinosorb S administered orally (gavage) at dose levels of 0 (polyethylene glycol 400 only), 100, 300 or 1000 mg/kg/day from day 6 to day 17 post-coitum (anticipated gestation period). The test material was delivered in a volume of 5 mL/kg, with daily adjustment made to correct for actual body weight. All animals

were continuously monitored for survival and clinical signs of toxicity. Study females were sacrificed on day 21 post-coitum and the uterine contents removed for examination.

All dams survived to sacrifice, with no apparent clinical signs of toxicity. There was no evidence of an adverse on the dams, with food consumption and weight body (gain) data shown to be consistent across all groups. All dams were free of macroscopic abnormalities at autopsy.

The test material did not appear to have an adverse effect of reproductive performance, with all mated females found to be pregnant. Confirmation of this result was seen in maternal reproduction data, where sex ratio, mean number of corpora lutea, implantation sites and % of pre- and post-implantation losses were similar between treatment groups and controls.

Examination of the foetuses did not detect evidence of treatment-related external abnormalities. There was an increase in the body weights of foetuses to females receiving 1000 mg/kg/day, while body weights of foetuses in the lower treatment groups were similar to controls. The body weights were 4.7, 4.7, 4.8 and 4.9 g for the control, LD, MD and HD groups, respectively, which although marginally greater was not significantly greater at the HD. Mean litters sizes were 11.6, 12.4, 11.4 and 10.8 for the control, LD, MD and HD groups, respectively. It was suggested that the slightly greater foetal body weight for the HD group could be linked to the slightly lower mean litter size, with the lower number of foetuses having greater potential nutrient exposure compared to other groups.

It was concluded that dose levels of 100, 300 and 1000 mg/kg/day would be suitable for the main pre-natal toxicity study in rats.

> Prenatal toxicity study with Tinosorb S in the rat (RCC Labs; report no. 681491; H. Becker & K. Biederman; 1998; GLP/QA-yes).

The purpose of this study was to investigate the effects of Tinosorb S on pregnant female rats and their developing embryos/foetuses. Groups (22/dose) of mated female Wistar rats had Tinosorb S administered orally (gavage) at dose levels of 0 (polyethylene glycol 400 only), 100, 300 or 1000 mg/kg/day from day 6 to day 17 post-coitum (gestation period). The test material was delivered in a volume of 10 mL/kg, with daily adjustment made to correct for actual body weight. All animals were continuously monitored for survival and clinical signs of toxicity. Study females were sacrificed on day 21 post-coitum and the uterine contents removed (under caesarean section) for examination.

All mated females survived to scheduled sacrifice. Clinical signs of toxicity were limited to an occurrence of soft faeces, which was identified as a common finding in animals treated orally with polyethylene glycol 400. This contention was supported by the finding that all animals (treatment groups and controls) produced soft faeces during the course of the study. Apart from this finding (soft faeces) animals were free of overt clinical signs.

At the autopsy of the dams, the type and frequency of findings were consistent across all groups and represented common pathology seen in this strain of rat. The low frequency and common nature of the changes indicated that the test material had no adverse effects on the tissue/organs

at the dose used in this study. It was noted that a female from the LD and MD groups, which were not pregnant, had dark red discoloured ovaries. Another female from the MD group had light brown foci in the left kidney. In the HD group, a female had the right uterine horn and ovary missing (reason for not falling pregnant) and another female had a light brown nodule on the left kidney. The control group was free of abnormal findings.

Reproduction data for this study were unremarkable. Mating resulted in 22, 21, 21 and 21 pregnant females from the control, LD, MD and HD groups, respectively. These numbers were used in the estimations of reproductive performance. Confirmation of this result was seen in maternal reproduction data, where sex ratio, mean number of corpora lutea, implantation sites and % of pre-implantation losses were similar between treatment groups and controls. An increase in post-implantation losses (%) and a reduction in viable foetuses (measured as % total foetuses/ implantation sites) in the LD and HD groups were evident (see table below).

Parameter	Control	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Post-implantation loss %	3.5% (0.5)	14.5% (1.7)*	6.0% (0.7)	9.0% (1.1)*
Foetal viability	96.5%	85.5%*	94.0%	91.0%*

Note: The value in the brackets in the post-implantation loss section of the table is the mean raw value for implantation loss. The values marked with the asterisk (*) were significantly different from the control value.

These effects were considered not to be treatment-related since there was no dose-relationship and the resultant values for these parameters fell within the historical control range of values for the laboratory.

Data on the foetuses indicated that the mean body weights, the sex ratio, and the results of external, visceral and skeletal examinations were consistent across all groups. There was no evidence of any treatment-related adverse effects on the development of the foetuses in this study. Body weights of all treatment groups were marginally higher than the controls. It was suggested that the slightly greater foetal body weight for the treatment groups could be linked to the slightly lower mean litter size, with the lower number of foetuses having greater potential nutrient exposure compared to the controls. External examination did not detect any foetal abnormalities. Skeletal examination detected agnathia (congenital absence of the lower jaw) in a control (1/274) and a MD (1/236) foetus. There were no abnormal findings in the LD (213) and HD (232) foetuses. Visceral examination found revealed cleft palate, microphthalmia (abnormal smallness of one or both eyes) and an absence of a tongue in the control and MD foetuses that had agnathia. Stedman’s medical dictionary noted that further abnormalities are often seen in animals displaying agnathia. There were no abnormalities in LD and HD foetuses detected during examination of the viscera.

It could be concluded that the test material did not interfere with the reproductive performance of dams or development of the embryos/foetuses at doses up to 1000 mg/kg/day administered during organogenesis in rats. Tinosorb S was not teratogenic in this study. A NEL of 1000 mg/kg/day was estimated for this study.

Uterotrophic assay in immature rats with Tinosorb S (Central Toxicology labs [UK]; report no. ZR1601; K. Twomey; 2002; GLP/QA-yes).

Groups (10/dose) of immature 20-21 days old female Wistar (Alpk:Ap$_f$SD) rats were given a single daily subcutaneous injection of Tinosorb S at doses of 0 (vehicle control), 250, 500 or 1000 mg/kg/day over a period of 3 consecutive days. A positive control (0.4 mg/kg 17β-estradiol) group was included in the study. The vehicle used in this study was arachis oil. General health and body weights of the animals were monitored on a daily basis. Approximately 24 hours after the end of the treatment period the animals were sacrificed and the uterus removed and weighed.

There were clinical signs of toxicity observed during the course of the study. Body weights of Tinosorb S (45.5-57.2 g) treated and positive control (45.5-55.0 g) animals were similar to the vehicle controls (45.6-56.0 g). Tinosorb S (0.023-0.025 g) did not have an effect on the uterine weights of the animals in this study at a dose up to 1000 mg/kg/day, when compared to vehicle controls (0.024 g). The positive control did induce a significant increase in the weight (0.107 g) of the uterus to validate the procedure.

There was no evidence of an uterotrophic effect following subcutaneous injection of Tinosorb S at dose up to 1000 mg/kg/day in immature female rats.

Androgen competitive binding assay with Tinosorb S (Central Toxicology labs [UK]; report no. CTL/024530; P. Lefevre; 2001).

In this assay, rat prostates were used as a source of cytosolic androgen receptors (AR). The prostate/s were removed from chemically castrated 8 week-old male Alpk:ApfSD rats and homogenised (in DMSO). Collected homogenate was incubated with appropriate concentrations of AR receptor antagonist, positive control and test agents, as well as the vehicle control. After incubation, the receptor-ligand complex was isolated and analysis of relevant receptor-ligand interactions determined.

The generated data showed that Tinosorb S had no activity at the AR receptors at the concentrations (up to $5x10^{-4}$ M) tested.

Oestrogen competitive binding assay with Tinosorb S (Central Toxicology labs [UK]; report no. CTL/024529; P. Lefevre; 2001).

In this assay, rat uteri were used as a source of oestrogen receptors (ER). The uteri were removed surgically from 21-25 day-old immature female Alpk:ApfSD rats, trimmed, weighed and frozen until the day of the assay. At the time of the assay, frozen uteri were homogenised (in buffer) and prepared for the assay. Collected homogenate was incubated with appropriate concentrations of ER receptor antagonist, positive control and test agents, as well as the vehicle control. After incubation, the receptor-ligand complex was isolated and analysis of relevant receptor-ligand interactions determined.

The generated data showed that Tinosorb S had no activity at the ER receptors at the concentrations (up to $5x10^{-4}$ M) tested.

Environmental impact studies

A series of studies on the effects of Tinosorb S on organisms within the environment were submitted for assessment. The following is a summary of the findings of these studies.

> Toxicity of Tinosorb S to Scenedesmus subspicatus in a 72-hour algal growth inhibition test (RCC Labs; study no. 672063; U. Memmert; 1998; GLP/QA-yes).

This study examined the effect of Tinosorb S on the growth of the green algal species Scenedesmus subspicatus. Tinosorb S was found to have a very low water solubility, which led to the generation of supersaturated stock suspension of a nominal concentration of 100 mg/L. This stock solution (maximum dissolved test material) was filtered and only the undiluted filtrate was added to the test medium containing the algal species. Analysis of the test medium found the concentration of the test material was 17 µg/L at the start of the assay.

Scenedesmus subspicatus and the test material were incubated together for 72 hours prior to assessing inhibitory activity. Tinosorb S had no inhibitory effect on the growth of Scenedesmus subspicatus after 72 hours at a concentration estimated to be well in excess of the normal water solubility limit of the test material. A LOEC (lowest concentration tested with toxic effects) for Tinosorb S could not be estimated as there was no evidence of toxicity.

> Toxicity of Tinosorb S to activated sludge in a respiration inhibition test (RCC Labs; study no. 672085; U. Memmert; 1998; GLP/QA-yes).

The inhibitory effect of Tinosorb S on the respiration rate of aerobic waste water micro-organisms of activated sludge was investigated over a incubation period of 3 hours. Concentrations of Tinosorb S used in this assay were 10, 32, 100, 320 and 1000 mg/L. Negative and positive control (3,5-dichorophenol) groups were included in the assay.

Tinosorb S had no significant inhibitory effect on the respiration rate of activated sludge after 3 hours incubation. Preparation of Tinosorb S for addition to the sludge required generation of supersaturated solution, which resulted in the test organisms being exposed to concentrations of the test material greater than its limit of water solubility and environmental concentration. The EC20 and EC50 values for Tinosorb S in sludge were >1000 mg/L nominal. The positive control had a mean EC50 value of 23 mg/L (range 5-30 mg/L).

> Acute toxicity of Tinosorb S to daphnia magna in a 48-hour immobilisation test (RCC Labs; study no. 672041; U. Memmert; 1998; GLP/QA-yes).

This assay examined the acute toxicity of Tinosorb S to daphnia magna in a 48-hour static test, which examines both the mobility and survival of the test organism. Tinosorb S was found to have a very low water solubility, which led to the generation of supersaturated stock suspension

of a nominal concentration of 100 mg/L for use in this assay. The concentration of Tinosorb S used was dictated by its solubility and amounted to 144 μg/L.

Tinosorb S was not toxic towards daphnia magna during an exposure period of 48 hours at a concentration that exceeded the water solubility limit of Tinosorb S. The level tested had no adverse effect on the survival or mobility of the test organism.

> Acute toxicity of Tinosorb S to Zebra fish in a 96-hour static test (RCC Labs; study no. 672028; U. Memmert; 1998; GLP/QA-yes).

The acute toxicity of Tinosorb S to Zebra fish (Brachydanio rerio) was determined in a 96-hour static exposure test conducted in accordance with OECD guidelines. Tinosorb S was found to have a very low water solubility, which led to the generation of supersaturated stock suspension of a nominal concentration of 100 mg/L.

Tinosorb S had no toxic effect on zebra fish during the exposure of 96 hours at a concentration at the water solubility limit of the test article. It was noted that LC50 and LOEC values could not be determined due to the absence of toxicity. Tinosorb S was tested at a concentration up to the water solubility limit of the test material.

Bio-accumulation test of Tinosorb S in carp (Institute of Ecotoxicity, Gakushuin Uni. Japan; Report no. G4-9915.C-102.CR; M. Takamatsu & N. Iizuka; 1999; GLP/QA-yes).

The aim of this study was to evaluate the potential bio-accumulation of Tinosorb S in carp (Cyprinus carpio). The procedure employed in this study conformed to the "method for testing the degree of accumulation of chemical substances in fish body", Japanese health standard. An amount of 3.00216 g of Tinosorb S was prepared (ground in mortar with chloroform) and added to water (temperature 24.4^0C) of a fish tank with a dispersing agent (polyoxyethylene hardened castor oil). Dispersion of the test material was described as good. A positive control substance (mercuric chloride) was included in a separate tank of fish. Viability assessment of the fish in either the test material or positive control tank over a period of 48 hours was conducted. Stability testing of the test material in the tank water was conducted at 0, 24 and 48 hours. A total of 25 fish were exposed to nominal levels (2 groups with 0.1 or 1.0 mg/L) of the test material, while a further 6 fish were exposed to the positive control agent.

The procedure to determine whether the test material bio-accumulated in the carp involved 8 weeks exposure to the test material in the tank water.

Evaluating viability of fish showed no deaths in the tank containing Tinosorb S, while all fish exposed to mercuric chloride died within the 48 hours exposure period. In the assessment of potential bio-accumulation, concentrations of the test material (0.101-0.103 mg/L; 0.948-0.981 mg/L) in the tank water were shown to approximate stated nominal concentrations. It was stated that the test material did not accumulate in the tissue of carp under the conditions described above.

Ready bio-degradability of Tinosorb S in a manometric respirometry test in activated sludge (RCC Labs; study no. 672096; U. Memmert; 1998; GLP/QA-yes).

Tinosorb S (100 mg/L) was investigated for its bio-degradability in activated sludge using a manometric respirometry assay (according to OECD guidelines) conducted over a 28 days test period. The activated sludge was from the aeration tank of a domestic waste-water treatment plant. Aniline was used as a procedure control in this assay.

Tinosorb S did not show any significant bio-degradability under the conditions described above. A mean bio-degradation value of 2.6% was determined for Tinosorb S over the 28 days study period, with a raw value range of zero to 2.7%.

In a toxicity control using both Tinosorb S and aniline (positive control), no inhibitory effect on the bio-degradation of aniline was determined, which reinforced that Tinosorb S had no inhibitory effect on activated sludge. Aniline baseline (control) effect on the activity was bio-degraded by a mean of 83.4% by day 14 and 86.7% by the end of the test.

OTC MEDICINES SECTION

ASSESSMENT OF NEW TOPICAL ACTIVE

Bisimidazylate

Sponsor:	Symrise Pty Ltd (formerly Haarmann & Reimer)
File Number:	2004/024808
TGAIN:	198431
Report Date:	November 2004
Evaluator:	s22 [redacted]

IDENTITY

INCI:	Disodium phenyl dibenzimidazole tetrasulfonate (DPDT)
AAN:	Bisimidazylate (proposed)
CAS Number:	180898-37-7
Chemical Name:	2,2'-(1,4-pehnylene)bis-(1H-benzimidazole-4,6-disulfonic acid, monosodium salt
Lab. Codes:	HR96/N00002;
Trade Name:	Neo Heliopan AP
Drug Class:	UVA filter
Empirical Formula:	$C_{20}H_{12}N_4O_{12}S_4Na_2$
Molecular Weight:	674
Appearance:	Yellowish powder
Solubility:	0.25% in water
Proposed Use:	UVA filter at a maximum concentration of 10%
Structure:	

SUMMARY OF TOXICOLOGICAL FINDINGS

Kinetic data single oral dose in rats	1.8-2.1% absorbed
Kinetic data single dermal dose in rats	0.1-0.3% absorbed
In vitro percutaneous assay with human skin	below detection
Acute oral toxicity in rats	LD50>2000mg/kg
Acute dermal toxicity in rats	LD50>2000mg/kg
Acute inhalation toxicity in rats	LD50>1870mg/cm^2
Primary skin irritation in rabbits	Non-irritating
Skin sensitisation (max. test) in guinea pigs	Non-sensitising
LLNA in mice	Unlikely sensitiser
Acute eye irritation in rabbits	Non-irritant
In vitro HET-CAM test	Slight response

s47 [redacted]

Phototoxicity in humans	Negative
Photoallergenicity in humans	Negative

13 weeks oral toxicity study in rats	NEL 695 mg/kg/day in males NEL 824 mg/kg/day in females

s47 [redacted]

Range finding teratology study in rats	NEL 1200 mg/kg/day
Developmental toxicity study in rats	NEL 1200 mg/kg/day

Genotoxicity Ames assay *in vitro*	Negative
Chromosomal aberration in human peripheral lymphocytes *in vitro*	Negative
V79-HGPRT forward point mutation assay in CH lung cells *in vitro*	Negative

s47 [redacted]

Introduction

This is an application to approve disodium phenyl dibenzimidazole tetrasulfonate (Neo Heliopan AP; proposed AAN bisimidazylate) for use an active ingredient (UVA filter) primarily in sunscreen preparations at a concentration of up to 10%. An initial data package was submitted in Europe (1998), but since then further studies have been conducted and these are included in the application to the TGA. The sponsor provided an assessment of Neo Heliopan AP addressing the AS/NZS2604:1998 definition of a UVA filter (complies to broad-spectrum requirement). Neo Heliopan AP was shown to fit the stated requirements for a UVA filter using the methodology described in the sunscreen standard (AS/NZS2604: 1998). Neo Heliopan AP was found to have an absorption spectra maxima at 340 nm.

s47

Kinetics

The data submitted included an ADME (absorption, distribution, metabolism & excretion) study for the oral, dermal and IV routes in rats, *in vitro* pig skin and rat skin dermal absorption (penetration) assays and an *in vitro* percutaneous absorption study using human skin. There were no plasma levels of bisimidazylate measured during the 13 weeks oral dietary toxicity study.

Data from the ADME study (using AUC data) in the rat showed that bisimidazylate was poorly absorbed from the GIT, with amounts absorbed in the order of 1.8-2.1% after administration of a single oral dose. Cmax was reached between 1-3 hours post-dosing and the elimination half-live was in the range of 7-9 hours. Dermal absorption in the rat was even less than after oral dosing,

with only 0.1-0.3% absorbed after application of a single dose. Cmax was reached between 6-8 hours post-dosing and the elimination half-life was in the range of 14-16 hours.

In vitro testing of dermal absorption in rat and pigskin reinforced the *in vivo* findings in rats, with absorption through rat skin (epidermis) shown to be formulation dependent (in rats). Testing for absorption (rat epidermis) of bisimidazylate from either a water in oil formulation or an oil in water formulation found no bisimidazylate (limit of detection 0.05%) following application of the water in oil formulation and 0.49% from an oil in water formulation. The value (0.49%) determined in this assay was used by the SCCNFP to estimate a margin of safety.

In the assay using pig skin (stated to be a good approximation to human skin), no bisimidazylate penetrated the skin with none found in the receptor fluid. Only trace amounts (0.2-0.3%) were found in the epidermis/dermis and it was stated that it was unlikely that this material would move deeper. Both formulations mentioned above were tested, with no difference (below limit of detection) in absorption of bisimidazylate in pigskin.

In the percutaneous absorption assay with human epidermis, absorption of bisimidazylate from two formulations produced similar results, with no penetration into the receptor from all (24) samples using the water in oil formulation and 22/24 samples from the oil in water formulation. Two samples provided a reading (<0.33%) thought to be due to contamination or measurement error. Overall, it was concluded that bisimidazylate did not penetrate human epidermis above limits of quantification.

Based on the lower absorption of bisimidazylate through human epidermis, when compared to absorption through rat skin used to estimate the margin of safety, the previously discussed (refer safety assessment) margin of safety could be justifiably greater for human exposure.

Safety assessment

The SCCNFP (see attachment 1) concluded that bisimidazylate is an ingredient that is safe for use in cosmetic products as an UV light absorber at a maximum concentration of 10%. Included in their assessment was a calculation of safety margin, which indicates that the margin of safety was 4700 estimated from the following information. An application amount of 10 mL (approx. 18 g) containing 10% bisimidazylate (1800 mg active applied), to a 60 kg person with an absorption of 0.49% = 8.82 mg (1800 mg x 04.9% absorbed). Systemic exposure dose per kg equals 0.147 mg/kg (8.82 mg/60 kg). The value for anticipated systemic exposure (0.147 mg/kg) was compared to the lowest NEL in the toxicity studies, which was 695 mg/kg/day for males in the 13 weeks feeding study. Their estimated margin of safety was 4700 (695/0.147 = 4728).

This is a simplification of the situation, which would be corrected if the systemic exposure (from oral kinetic study in rats) associated with the NEL (695 mg/kg/day) was compared to the estimated systemic exposure likely to achieved by applying bisimidazylate (in sunscreen) to the skin. This would be a better approximation of related exposures, in the absence of AUC data for human exposure (animal exposure based on AUC data). Therefore, the systemic exposure associated with toxicity study generating the NEL was approximately 2% of 695 mg/kg/day, which would result in 13.9 mg/kg/day. The anticipated exposure following normal usage was

estimated as 0.147 mg/kg, which would be 95 fold less (safety margin) than the NEL for the animal toxicity studies.

It must be remembered that there was no evidence of treatment-related toxicity at a dose of 695 mg/kg/day (highest dose used) in male rats, which means the actual threshold dose for toxicity could be considerably higher. It was noted that there were negligible (below limit of detection) amounts of bisimidazylate absorbed through human epidermis *in vitro*, while measurable amounts were absorbed through rat skin. This suggests that dermal absorption from topical application in humans would be less than absorption through rat skin. Estimation of the safety margin was based on absorption through rat skin and as such could be underestimating the safety margin (possible >95).

Interaction with other UV filters

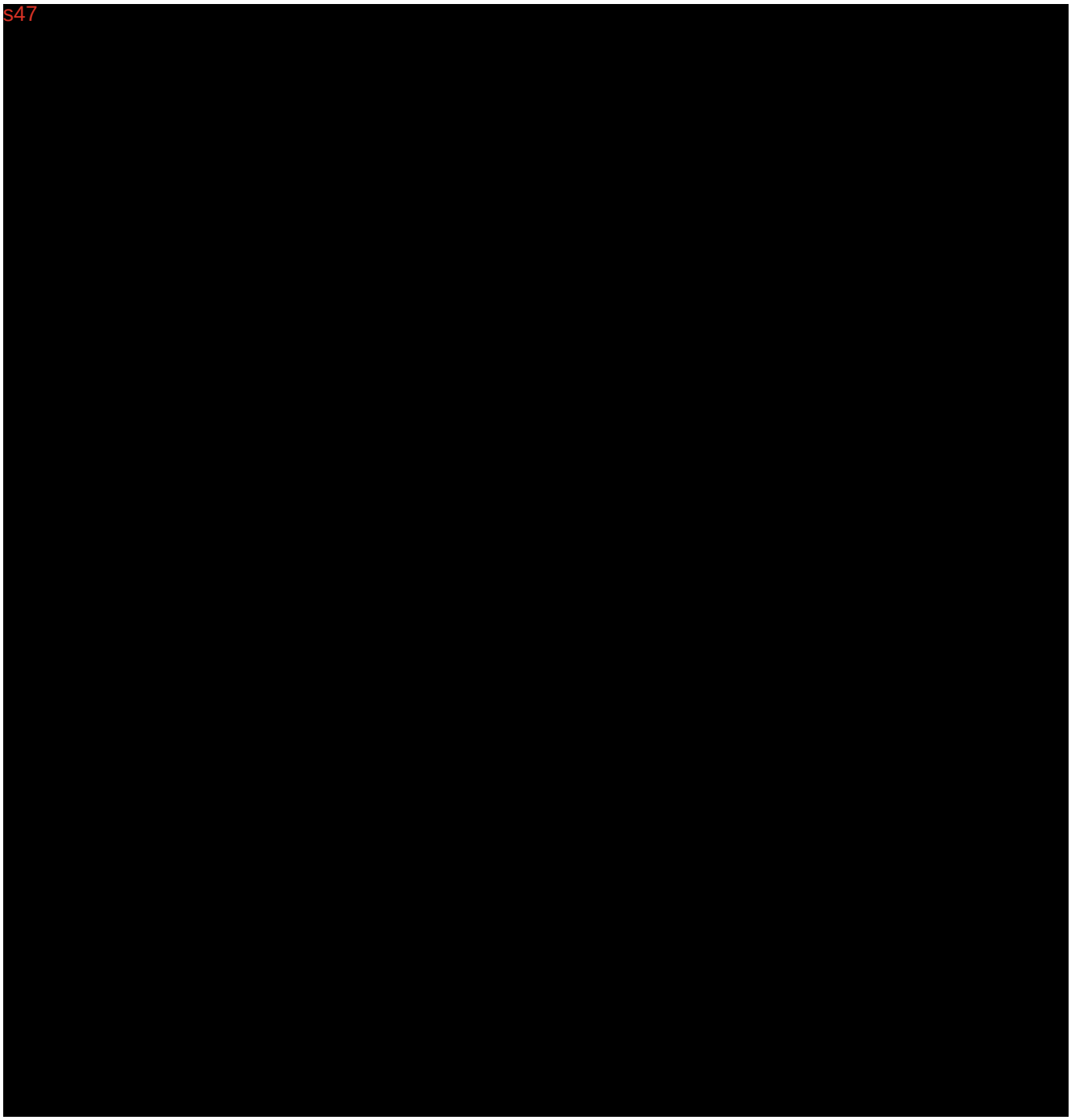
s47

Local tolerance

In animal studies, bisimidazylate (all related formulations) was not a primary skin or eye (*in vivo* and *in vitro* assays) irritant in the rabbit. Contact hypersensitivity (maximisation test) for formulations (10% aqueous, 10% emulsion, vehicle only) containing bisimidazylate was assessed in the guinea pig, with results showing no evidence of hypersensitivity under sensitised conditions. Included as a measure of skin sensitisation potential was an assessment of bisimidazylate (>98% & 10% aqua.) *in vitro* local lymph node assays (LLNA), with results suggesting the test formulations were unlikely to possess sensitisation potential.

s47

s47



General toxicity

Acute oral and dermal toxicity studies in rats showed that bisimidazylate has low acute toxicity, with LD50 values of >2g/kg for both routes of administration. A LD50 value of >1870 mg/m^3 was estimated for an acute inhalation toxicity study in rats.

Repeat-dose toxicity was assessed in a 13 weeks dietary study in rats, which included a 4 weeks drug-free recovery period. Doses of up to 10000 ppm resulted in maximum intakes of 695 and

824 mg/kg/day in males and females, respectively. Animals remained in good health throughout the study period, with the only death (female control) occurring as a result of an error in blood collection. Body weights were similar across all groups and food intake was slightly elevated in animals in the treatment groups. Haematology and clinical chemistry were unremarkable, with any observable changes seen as transient, lacking a dose relationship and/or only occurring in one sex.

Analysis of organ weights and tissue pathology was unremarkable, with any observable changes seen as transient, lacking a dose relationship and/or only occurring in one sex. Scrutiny of data for reproductive organ/tissues revealed no adverse effects as a result of exposure to the test material. Ovaries and testes weights were similar across all groups. Examination of individual data for tissues such as testes, epididymides, prostate, seminal vesicles, ovaries, oviducts, uterus and vagina were in general unremarkable. There was a low incidence (not statistically significant) of dilation of uterine horns in high dose females, which was the only occurrence of this change but it was considered not to be of biological significance. Overall, an estimate of the NEL for this study was 10000 ppm in both sexes of rat.

Two studies (dose-range finding & main) were submitted that examined the potential adverse effects of bisimidazylate on the development of the rat foetus during organogenesis. In the dose-range finding study, there was no evidence of any adverse effects on dams (no maternal toxicity) or foetal development at doses up to 1200 mg/kg/day delivered during organogenesis. Reproductive parameters were similar for all groups, indicating the test material behaved like the vehicle in this assay. It was determined that an appropriate dose range for the main study would be 400-1200 mg/kg/day.

In the main study, there was no evidence of any adverse effects on dams (no maternal toxicity) or foetal development at doses up to 1200 mg/kg/day delivered during organogenesis. Reproductive parameters were similar for all groups. Assessment of any potential teratogenic activity of the test material showed that there were no instances where the frequency of an abnormality/anomaly in foetuses exposed to the test material were greater than control frequencies. The estimated NEL for both maternal toxicity and developmental effects was the highest dose used in this study (1200 mg/kg/day). There was no evidence that bisimidazylate interfered with processes involved with the maturation of tissue/cells.

Genotoxicity

There were 5 *in vitro* studies conducted using both bacterial and mammalian cell cultures. These investigations included an Ames assay, a chromosomal aberration assay in human peripheral lymphocytes, a V79-HGPRT forward point mutation assay in CH lung cells, an Ames & E. coli assay and a chromosomal aberrations assay in CHO cells. There were no *in vivo* genotoxicity assays presented with this submission. End-points covered in these assays were reverse and forward point mutations (base pair substitutions, frameshift) in bacterial and mammalian cells and clastogenic activity (breakage of chromosome).

s47

The data package supporting this application did not include any *in vivo* genotoxicity studies.

s47

s47 [redacted]

Bisimidazylate did not generate a positive response in any of the genotoxicity or photogenotoxicity assays (*in vitro* only) provided, indicating that it is unlikely to initiate a neoplastic event through a genotoxic mechanism. s47 [redacted]

There were no data on repeated dermal exposure to bisimidazylate, which could have been useful for assessing changes such hyperkeratosis or acanthosis that could result from prolonged exposure to bisimidazylate. Information in the literature noted that skin tumour promoters consistently cause sustained dermal hyperplasia (acanthosis) in mouse skin models. Furthermore, chronic skin irritation and inflammation could also be relevant to topically substances having a potentially adverse effect leading to neoplastic events. In rats, an association between macroscopic/microscopic signs of chronic irritation/inflammation and cancer has not been substantiated. However, it was shown that bisimidazylate did not cause significant skin irritation in humans in repeat insult testing (up to 9 applications over 3 weeks) and was classified as a non-irritant to animal skin.

RECOMMENDATION

The sponsor has submitted a data package containing studies on bisimidazylate which conform to GLP standards and OECD or ICH guidelines. Bisimidazylate is a UV filter that has been on the accepted list of UV filters in the EU since 2000 at concentrations up to 10% in products.

The features of bisimidazylate are:-

Systemic exposure in rats following administration via the oral (1.8-2.1%) and dermal (0.1-0.3%) routes was very low, and *in vitro* percutaneous penetration through human skin was below the limit of detection (0.05%).

s47 [redacted]

s47 [redacted] did not interfere with androgen or oestrogen receptors and was not uterotrophic in the rat.

s47 [redacted]

The likelihood of bisimidazylate being a dermal carcinogen would be considered low.

The preceding information indicates that the likelihood of bisimidazylate causing systemic or dermal toxicity is low. On this basis, the use of bisimidazylate in listed/registered sunscreen products at concentrations up to 10% is proposed.

The advice of the committee is requested

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s47

Document 9

s22 [redacted]

OTCMS
August 2005

OTC MEDICINES EVALUATION SECTION – TGA

SAFETY EVALUATION OF NEW SUNSCREEN ACTIVE

Drometrizole Trisiloxane

Table of Contents

OTC MEDICINES EVALUATION SECTION – TGA

SAFETY EVALUATION OF NEW SUNSCREEN ACTIVE

Drometrizole Trisiloxane

Sponsor:	L'Oreal Australia Pty Ltd
Consultant:	Sue Akeroyd & Associates
Proposed AAN:	Drometrizole Trisiloxane
Trade names:	Mexoryl XL; Silatrizole; G4375
CTFA/INCI name:	Drometrizole trisiloxane
Chemical name:	phenol, 2-(2H-benzotriazol-2-yl)-4-methyl-6[2-methyl-3-[1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]disiloxanyl]propyl
Empirical formula:	$C_{24}H_{39}N_3O_3Si_3$
CAS No.:	155633-54-8
Molecular weight:	501.855
Chemical class:	Hydroxyphenyl benzotriazole derivative
Appearance:	White to yellowish crystals, practically odourless
Boiling point:	530^0C
Melting point:	180^0C
Density:	0.94 g/cm^3
Water solubility:	Practically insoluble (0.04 mg/L at 20^0C)
UV Spectrum:	λmax_1 = 303nm ε_1 = 16200 mol.L^{-1}.cm^{-1} E1% = 323
	λmax_2 = 344nm ε_2 = 15500 mol.L^{-1}.cm^{-1} E1% = 309
Purity:	Minimum 98%; it was noted that most impurities may originate from reagents and intermediate reaction products (organic impurities) and solvents (methanol, isopropanol).

It was stated that trace analysis of volatile compounds was performed on a sample batch (no. DG004), using headspace gas chromatography. Results for the given sample indicated that no solvent residues were detected (less than 100 ppm). All of the impurities are present at concentrations of no more than 1%.

Structure:

Introduction

Drometrizole Trisiloxane is a new UV filter proposed for use in listed sunscreen products. The sponsor noted that most of the studies presented for evaluation refer to G4375, which is L'Oreal's code representation for drometrizole trisiloxane. The substance is a hydroxyphenyl benzotriazole derivative. Derivatives of 2'-hydroxy-2-phenylbenzotriazole (Drometrizole) have been extensively used in polymer photo-protection for over 40 years and their photochemistry has been extensively studied. The very short lifetime of their excited states explains their activity and duration of photo-protection. These molecules have a unique planar structure with an intramolecular hydrogen bond, which allows for a rapid non-radiative deactivation process through a forward/backward intramolecular transfer. The cycle of excitation/electronic deactivation/equilibration in its vibrational energies is accomplished within picoseconds. Drometrizole trisiloxane differs from the more extensively studied drometrizole, by an alkyl-siloxanyl substitution on the 3'-position, which has a tendency to strengthen the intramolecular hydrogen bond and further stabilise the molecule in polar media (sponsor statement).

Regarding the data package, all appropriate safety studies were performed under Good Laboratory Practice (GLP), and in most cases following the relevant OECD or ICH Guidelines.

International status

Drometrizole trisiloxan is included in the EC list (Directive 76/768/EC) of UV filters, "which products can contain at a maximum concentration of 15%" (ref. 53). The entry onto the list was dated 15/9/1998.

International regulatory comment

Included in the data package was a copy the "Opinion of the Scientific Committee on Cosmetology on drometrizole trisiloxane". The SCC provided an opinion that, "the compound seems to have low acute and subchronic toxicity. There is no evidence from animal experiments that the compound is an irritant to skin or mucous membranes, nor is there any evidence of allergenic or photoallergenic activity. Tests for mutagenicity and photomutagenicity are negative. A test for teratogenic activity in the rat is negative. In a test for percutaneous absorption carried out in human skin ex vivo, the amount absorbed was at most 0.8% of the amount applied" (ref. 52). Included in the SCCNFP report (p14) was a graphical representation of the absorption spectra of drometrizole trisiloxane, which showed it would be effective as a filter across the UVA range.

Interaction data and photodegradation study for Mexoryl XL

The following information identifies the integrity of the parent molecule in the toxicity studies presented for evaluation and is not an assessment/evaluation of the stability of either drometrizole trisiloxane or a formulation containing drometrizole trisiloxane.

General data were presented that detailed thermal degradation, degradation in acidic and basic environments at 20^0C and 40^0C, degradation after storage for 9 months at ambient temperature

(protected from light) and photodegradation (see below). It was stated that drometrizole trisiloxane was stable under the conditions described above (French copy of data held).

In a 5 page study (ref.55), the photodegradation of Mexoryl XL was examined using experimental conditions approximating the potential conditions of use by the public. Mexoryl XL has been formulated at a concentration of 4% w/w (active G4375, batch RF004) in a non-ionic emulsion containing glycerol (mono- and di-stearate), triglycerides of saturated C8-C12 fatty acids, polydimethylsiloxane, cetyl alcohol, cetyl stearyl alcohol/cetyl stearyl and water. Prepared films of the test formulation were exposed to light generated by a solar simulator (2500 W Xenon lamp) to deliver a higher UV irradiance than would occur with the summer sun. Samples were exposed to the lamps without being heated beyond a temperature of 40^0C. It was noted that exposure in the conditions established for the test were equivalent to a dose of 3 times the intensity of the sun; 1 hour exposure was equivalent to 3 hours exposure to the sun. Extraction and analysis (chromatographic procedure) of the test material were carried out after exposure to determined the stability of the formulation/test material. Limited data (1 table) were presented with the report, which indicated that Mexoryl XL is stable after an equivalent 3 hours exposure to the sun. The percentage of test material in the exposed and non-exposed films did not vary significant (4.59±0.113% vs 4.61±0.069%, n=8).

A study (ref. 33) on the degradation of G4375 in rat and human plasma has been evaluated and is described in the pharmacokinetics section of this report. G4375 was found to be unaffected in human and rat plasma at concentrations up to 1000 ng/mL following incubation for up to 24 hours.

Adverse effects

The sponsor stated that no known, potential or suspected adverse effects relevant to drometrizole trisiloxane have been noted in any of the pharmacological and toxicological and human studies conducted and presented in this submission.

A further document (see attachment) was submitted that presented cosmeto-vigilance data on Mexoryl XL from the EU (dated Sept. 2002). Cosmeto-vigilance was described as a post-marketing system (established in 1979) with the objective of collecting, analysing and evaluating undesirable events on human health reported by consumers.

From an assessment of sales over a period of 3.5 years, the author of this document concluded that an intolerance incidence of <1/750000 units sold is very low for cosmetic leave-on products. These results suggest that Mexoryl XL has no or a negligible potential to produce adverse effects (on skin) in humans. It was pointed out that a cosmetic product can contain a mixture of numerous excipients and actives (in sunscreens in particular), so to single out Mexoryl XL as the cause of the adverse effect is not justified without specific analysis. It was not mentioned whether specific analysis had been carried out to identify the cause of adverse effect in affected individuals from Mexoryl XL containing products.

It should be noted that the cosmeto-vigilance post-marketing program is a company system and does not involve regulatory agency monitoring. The effectiveness of such a scheme may be limited.

ASSESSMENT/SUMMARY

Drometrizole trisiloxan is included in the EC list (Directive 76/768/EC) of UV filters which products can contain at a maximum concentration of 15% (ref. 53). It has been on the market in Europe for approximately four and a half years.

Pharmacokinetic/toxicokinetic

Studies analysing plasma samples obtained from rabbits, rats and mice revealed measurable levels of drometrizole trisiloxane were achieved following oral or dermal administration. However, plasma concentrations following oral or topical dosing were consistently very low (<1%) for all species. In a key rat study, it was apparent that plasma levels of drometrizole trisiloxane were lower following oral dosing when compared to topical application. In this study, radioactivity recovered in the urine after oral dosing accounted for 0.04-0.05% of the administered dose, while radioactivity recovered in the urine after topical dosing accounted for 0.31-0.33% of the administered dose.

In a (1000 mg/kg/day PO, 13 weeks) toxicity study in rats, the stability and impurity profile of an out-of-date sample of drometrizole trisiloxane (in-date at time of study) complied with the established standard and was found not to influence the analysis of plasma samples from this study. Analysis showed that 99.18% of the test sample was the parent substance, while total impurities (5) amounted to 0.8%. The impurity profile determined in this study was consistent with the known profile. Determination of drometrizole trisiloxane in plasma samples, taken at weeks 4 and 13 of dosing, was possible although the levels were low. Levels measured in males and females at both time points did not vary significantly, which would indicate no sex difference or likely accumulation in rats.

There were further studies that examined the stability of drometrizole trisiloxane in rat and human plasma, as well as *in vitro* metabolism by rat hepatocytes and hepatic microsomes from rats and humans. Drometrizole trisiloxane was found to be stable in rat and human plasma at 1000 ng/mL (similar to concentrations seen in kinetic studies), and incubation of drometrizole trisiloxane with rat and human microsomes produced on analysis one peak identified as the parent compound. This indicates metabolism of the parent molecule was limited or unlikely.

An *in vitro* test for percutaneous absorption using human skin *ex vivo*, found that the amount of test material absorbed was approximately 0.8% of the amount applied to the skin. The SCCNFP used this figure to determine/calculate a margin of safety for the topical use of the test material. The calculation assumed that 18 g of a sunscreen formulation would be applied in normal use (reasonable assumption). A maximum concentration of 15% (EU accepted standard)

drometrizole trisiloxane in a sunscreen could result in 2700 mg (15% of 18000 mg) applied to the skin. If 0.8% drometrizole trisiloxane is absorbed then a systemic burden would be 21.6 mg (0.8% of 2700 mg). Assuming an average body weight of 60 kg would lead to an estimated dose of 0.36 mg/kg (21.6 mg/60 kg) drometrizole trisiloxane. Using 60 kg as an average weight is appropriate; it is likely that in smaller people and children less would be applied on a reduced body surface area.

The calculated margin of safety depends on the No Observable Effect Level (NOEL) employed; a NOEL of 1000 mg/kg/day would give a margin of 2700 (1000/0.36), while a NOEL of 300 mg/kg/day would give a margin of 800 (300/0.36). This calculation appears to be based on an anticipated systemic exposure equal to the maximum administered dose (up to 1000 mg/kg/day), and does not consider the bioavailability of drometrizole trisiloxane from either the oral or dermal routes.

Interpretation of the toxicity studies (predominantly in rat) is confounded since an oral dose of 1000 mg/kg/day (approximately 0.1% absorbed) would result in an absorbed dose of about 1 mg/kg/day. Compare this with an absorbed dose of 0.36 mg/kg predicted as occurring in humans when they apply 18 g of sunscreen containing 15% drometrizole trisiloxane. It is acknowledged that a straight comparison of doses (mg/kg) is not the most accurate way to assess comparative systemic exposures (human vs rat), which should be made on a comparison of AUC data from both species. However, the only exposure data provided were for rats; no human exposure data were available or presented.

Interestingly, the value for human percutaneous absorption of 0.8% for drometrizole trisiloxane could be higher than the real rate of absorption, which would suggest a larger safety margin is applicable. This belief is based on the measured/calculated dermal absorption of 0.3% drometrizole trisiloxane in rats and the fact that rats have typically 2- to 5-fold higher percutaneous absorption values than humans (from Skin Barrier, Principles of Percutaneous Absorption, H. Schaefer & T. Redelmeier, Karger press, p120, 1996). Furthermore, two studies (refs. 28 & 29) measuring *in vitro* percutaneous absorption using human skin came up with values of <0.5% and 0.32%; it is not apparent where the value of 0.8% percutaneous absorption used by the SCCNFP for their estimation of safety margin comes from? It would appear that the actual human dermal absorption is lower than 0.8% of dose following dermal application.

The preceding discussion highlights the importance of understanding the toxicity issues associated with the administration of drometrizole trisiloxane in repeat-dose and reproductive oral toxicity studies (in rats). An analysis of the observed toxic effects from these studies (for oral and dermal routes) and their relevance to treatment with drometrizole trisiloxane is important to established appropriate NOEL's and safety margins.

Interaction with other UV filters

There were no specific studies examining possible interactions with other filters. However, stability/recovery data showed that drometrizole trisiloxane is very stable when formulated. It could be that drometrizole trisiloxane would be the only filter in a product since it appears to have >50% UV absorbance across a wide wavelength range of 290 to 380 nm (from graphical

presentation in SCCNFP report). This usage profile is supported by a sponsor statement that, “it may be used alone or in combination with other UV absorbers and reflectors up to 15% w/w in formulations. In general, concentrations of 2 to 4% are used in formulations”.

Lack of specific interaction data with other UV filters could be justified by the stable (and photostable) nature of drometrizole trisiloxane, which was not metabolised in biological systems and was not degraded in acidic or basic conditions at up to 40^0C.

Local tolerance

Drometrizole trisiloxane was classified as a non-irritating agent to the rabbit eye. There was evidence of slight reddening of the conjunctiva and slight chemosis, which was linked to a direct physical irritant effect of placing 0.1 g of a solid into the eye (not rinsed during testing). A skin irritation study in rabbits found drometrizole trisiloxane (500 mg for 4 hours under semi-occlusive dressing) had a primary irritation score of zero indicating it was not a primary skin irritant. A 2 weeks skin tolerance test in guinea pigs with drometrizole trisiloxane (0.04 g of 85% solution in corn oil) found no adverse effects were detectable; assessment included microscopic evaluation of application site. Therefore, animal testing showed that drometrizole trisiloxane was not likely to be a skin irritant.

Several animal studies examining the sensitisation potential of drometrizole trisiloxane were presented in the data package. In a Magnusson/Kligman maximisation test using 20 guinea pigs, a response classified as weak was measured in 1 animal only at the highest challenge concentration of 80% (not seen at lower concentration of 60%). It should be noted that this test procedure sensitises the animals to enhance their responsiveness. In a second maximisation study using 20 male guinea pigs, a challenge dose of 85% drometrizole trisiloxane in corn oil did not induce a positive response in any animal indicating the test material was a non-sensitiser. In a third study in 20 female guinea pigs using a modified Buehler assay (not maximisation assay) and a non-current positive control (α-hexylcinnamaldehyde) validation, induction and challenge doses of 85% drometrizole trisiloxane in corn oil were applied using a Hill Top chamber saturated with the test material. Assessment confirmed that there was no sign of skin irritation during the induction phase and no sign of a skin reaction during the challenge phase. Method validation had been supported by a 58% positive reaction rate for the positive control agent in assays using the same protocol. Based on the weight of evidence it was determined that drometrizole trisiloxane was not a sensitising agent in the animals models studied.

Examination of photoallergic potential was also carried out in 2 studies both using guinea pigs. Combinations of induction with drometrizole trisiloxane and UV irradiation followed by challenge were carried out. Results indicated that at concentrations up to 85% drometrizole trisiloxane was neither phototoxic nor photosensitising in the guinea pig.

Data from studies in humans were also presented. Human repeat-insult patch testing using 47 subjects (25 identified as atopic) generated the following findings. A total of 35/47 subjects showed no evidence (no visible relevant reaction) of skin irritation during induction, while the remaining 12 had slight but distinct erythema at 1 or 2 of the 9 assessment points only. These reactions were described as minimal and infrequent (random, after no more than 2 of 9

applications) in occurrence indicating G4375 (in acetone) could be described as a marginal skin irritant. It is possible that the use of acetone as the vehicle could have enhanced the skin reactivity of G4375 or caused a reaction of its own. It was noted that fewer individuals (5/47) showed any reaction during the challenge phase than was evident during the induction phase. These 5 subjects had marginal responses that were not evident at both times challenge responses were assessed. It was determined that drometrizole trisiloxane was not a skin sensitiser in a variety of subjects identified as normal and atopic.

Assessment of the phototoxicity (n=12) and photosensitisation (n=30) of drometrizole trisiloxane (15% in sunscreen formulation) in humans included evaluation of the formulation vehicle. In the phototoxicity study results revealed no skin reactions for formulation plus drometrizole trisiloxane and the vehicle alone. In the photosensitisation study, data presented for the induction period showed a protective effect of the sunscreen formulation, as evidenced by lower scores compared to sites exposed to the vehicle or left untreated. A similar pattern was seen for the challenge, with lower scores for the sunscreen formulation (approximating non-irritated skin). It was determined that the sunscreen formulation and its vehicle (minus active) did not induce photoallergic reactions under the conditions described above.

Studies (total subjects approx. 60) examining skin tolerability (cosmetic properties/acceptability) and comedogenic potential of drometrizole trisiloxane (at 10% in formulation) were presented for evaluation. In one study the product identified as sun cream appears to be acceptable as a cosmetic (ref. 38). Measurement of comedones and microcytes were used to evaluate the potential comedogenicity of sun cream 427184 (with 10% active). Application of the test material resulted in alleviation of comedones in 12 cases and microcytes in 6 cases. Therefore, the sun cream containing 10% drometrizole trisiloxane was regarded as highly acceptable and was seen as not possessing comedogenic potential.

Overall, the likelihood of drometrizole trisiloxane having an adverse local effect on normal human skin appears remote. The individual study sizes were not large (n=12-47), but an absence of positive reaction across all studies is reassuring. Secondly, negative results for skin sensitisation studies (humans and guinea pig) indicate negligible interaction of the test material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and, potentially, to DNA.

Acute/repeat dose toxicity

Acute oral and dermal toxicity studies in rats and mice showed that drometrizole trisiloxane has low acute toxicity (LD50 >2 g/kg), which is understandable when taking into consideration the very low systemic exposure following either oral or dermal administration of drometrizole trisiloxane. Intraperitoneal (IP) administration to rats produced evidence of toxicity, with LD50 values for rats of 563 mg/kg in females and 2000 mg/kg in males. In mice, IP dosing resulted in LD50 values of 1200 and 2000 mg/kg in females and males, respectively. Discolouration of the lungs appeared to be a common finding in both species, while adhesion of the liver lobes and/or adhesion of the intestines with the liver and the skin were observed. These effects would possibly be linked to direct contact with the test material and these organs in the abdominal cavity following IP injection. Fluid was seen in the thoracic cavity or in body cavities. Overall, there

was no obvious reason for the pronounced difference in LD50 values for males and females of both species. Interestingly, the acute toxicity of drometrizole trisiloxane would be considered moderate to low even when it bypasses the apparent absorption barriers of the GIT and skin.

Repeat dose oral toxicity studies of 14 days to 26 weeks duration in rats with drometrizole trisiloxane (100-1000 mg/kg/day) were in general unremarkable. In the 13 weeks study, fluctuations in some clinical chemistry parameters (plasma glucose, total cholesterol, plasma sodium, creatine kinase activity and plasma protein fractions) were observed, but these were often seen only in one sex or at one time point and were not dose-related. None of these changes were observed after a 4 weeks recovery period. Organ weight analysis, gross pathology and histopathology were unremarkable providing no apparent link/association to the clinical chemistry changes noted previously.

In the 26 weeks oral toxicity study, changes detected in clinical chemistry parameters included observations that plasma glucose levels were lower in mid-dose females (19%) and high-dose (up to 25%) males and females. Changes in glucose levels only reached significance at the week 26 analysis, but plasma glucose levels were consistently lower across the duration of the study in treated groups when compared to controls. Fluctuations in glucose of the amount described above may not be considered biological significant since the normal range varies over a range of 60-100 mg/100 mL (ref. Interpretation of diagnostic tests; J. Wallach, 3rd ed.). Reductions in glucose levels can be linked to pancreatic disorders and hepatic disease, amongst other problems. General clinical chemistry data and both macro- and microscopic examination of tissues/organs did not reveal any abnormality with either the pancreas (not assessed in organ weight analysis) or liver. It was noted that food consumption was transiently and marginally depressed in some of these animals (body weights not significantly depressed), which was possibly due to the method (gavage) of administration of the test material. A slight reduction in food intake and the presence of a non-calorie supplement (test material in solution) may have resulted in a slight reduction in glucose in the plasma?

Sodium and chloride levels were slightly (2-4%), but significantly elevated (not dose-related) in both sexes at week 6, however, this effect was not seen at 12/13 or 26 weeks. It was indicated that these changes were considered not to be of toxicological significance. Inorganic phosphate was slightly (up to 15%) elevated in males at week 26 at the mid- and high doses, but not at earlier analysis times at any dose; the observed change was not dose-related. Inorganic phosphate was elevated (up to 34%) in females at week 13 at the mid- and high doses, and at the mid-dose at week 26, but none of these changes were dose-related. Fluctuations in inorganic phosphate of the amount described above may not be considered biological significant since the normal range varies over a range of 3.0-4.5 mg/100 mL (ref. Interpretation of diagnostic tests; J. Wallach, 3rd ed.).

In the SCCNFP report (opinion document), their analysis of the toxicity studies concluded that the no observed effect level was 300 mg/kg/day, but the no observed adverse effect level was 1000 mg/kg/day. This indicates that the changes seen in the studies were of no toxicological significance, which is consistent with the preceding discussion. It was stated in a summary of the data that the effects were minor in degree and are suggestive of metabolic adaptations, or could also associated with the presence of a high concentration of stable molecule (non-nutritive) in the

gut. Effects seen throughout the repeat-dose toxicity studies were either seen only in one sex, were not dose-related, were observed at limited time points, were not linked to other changes (pathology) reflective of toxicity and were not consistently seen across a majority of studies. The dose that would cause toxicity in the rat or mouse had apparently not been reached (above 1000 mg/kg/day).

In a 13 weeks dermal toxicity study in mice with drometrizole trisiloxane (50 µL/mouse of 15% solution), was without any significant signs of toxicity. Macroscopic and microscopic examination of the test and control groups did not reveal any significant treatment-related abnormalities. Analysis of the histopathology showed a marginal increase in the incidence of epidermal hyperplasia in females (control 2 vs treated 5). It was noted that this finding was limited to females (in males zero incidence for treated animals and controls) and was classified as no more than minimal. An increase in the incidence of endometrial gland hyperplasia in the uteri of treated females was detected, but this was considered to be a hormonal dependent event controlled by the oestrous cycle and not due to the test agent.

It is important to note that conducting additional repeat-dose toxicity studies at higher doses than 1000 mg/kg/day would probably be counter productive, since kinetic data showed that increasing the dose does not lead to a relative increase in exposure (non-linear kinetics based on AUC data from rabbits). AUC values were 10.9 and 17.9 (µg.h/mL) for doses of 100 and 1000 mg/kg/day, respectively.

Reproductive toxicity/hormonal activity

In a series of studies, potential adverse effects of drometrizole trisiloxane on androgenic activity (rat), embryo/foetal development (rat and rabbit), fertility (rat) and pre-/post- development (rat) were presented for evaluation. In castrated male rats, drometrizole trisiloxane (up to 1000 mg/kg/day) did not possess androgenic activity. In females (results from 26 weeks oral toxicity study), endocrine disruptor (oestrogenic activity) activity of sunscreen actives was the focus of a recent study from Europe. In this study (26 weeks study in rats), there was no direct measurement of hormone levels to determine whether a change was induced by G4375. However, organ weight (defacto rat uterotrophic assay) analysis did not show any increase in the size of the rat uterus in this study. It was noticed that a trend toward smaller uterus and ovary weights was evident. Furthermore, gross and microscopic examination of the uterus and ovaries of study animals revealed similar spontaneous findings across all groups.

Developmental studies in rats treated with drometrizole trisiloxane (up to 1000 mg/kg/day PO) did not show any apparent adverse effects/abnormalities in foetuses. It was also apparent that there were no adverse effects on reproductive parameters during the course of the studies. In rabbits, an initial dose-ranging study was devoid of any overt signs of maternal or foetal toxicity (including abnormalities) at oral doses of up to 1000 mg/kg/day drometrizole trisiloxane. In the main study in rabbits, there were no adverse effects on maternal condition at doses of up to 1000 mg/kg/day G4375 delivered by gavage during organogenesis (days 6 to 18 of gestation) in the Chinchilla rabbit. In general, the occurrence of either external, visceral or skeletal abnormalities were similar across all groups, with no evidence of dose-relationships and the majority of observed frequency of changes falling within historical control values. However, the incidence of

absence kidney/s and ureter/s was just outside the historical range and occurred in uncompromised (no evidence of maternal toxicity) animals. This was considered an equivocal result for developmental changes in the Chinchilla rabbit. In a repeat study to investigate the equivocal finding of an absence kidney/s and ureter/s, numbers of foetuses examined were 212, 224 and 285 at 0, 300 and 1000 mg/kg/day, respectively. There was no evidence of the previously identified finding of missing kidney/s and ureter/s seen in the same strain of rabbit over the same dose range (300-1000 mg/kg/day); dams in this study remained in good health during the course of the study. The initial finding of missing kidney/s and ureter/s was considered to be of no relationship to treatment with drometrizole trisiloxane.

In general, fertility and pre-/post-natal development studies conducted using rats with drometrizole trisiloxane (up to 1000 mg/kg/day PO) were unremarkable. It was noted that dams did have a slightly reduced food consumption at limited times during the course of the studies, but this had no effect of fertility, reproductive performance or foetal development and therefore considered to be an insignificant effect. Marginally reduced food consumption had been seen rats in oral toxicity studies of up to 26 weeks duration. The method of dose administration was gavage, which was common for all rat studies including reproductive toxicity studies. In the discussion of the oral toxicity studies it was suggested that the effects (includes reduced food consumption) were minor in degree and are suggestive of metabolic adaptations, or could also associated with the presence of a high concentration of stable molecule (non-nutritive) delivered into the gut. The SCCNFP arrived at a similar conclusion and accepted a NOEL of 1000 mg/kg/day for reproductive effects (includes developmental effects) of drometrizole trisiloxane in rats and rabbits.

A NOEL of 1000 mg/kg/day was estimated for reproductive toxicity.

Genotoxicity

Genotoxicity was examined *in vitro* in bacterial and mammalian cell systems, as well as *in vivo* in mice. Testing included reverse mutation assays (3) in S. typhimurium and E. coli, a gene mutation assay using Chinese hamster ovary cells, a chromosomal aberration assay in Chinese hamster V79, a micronucleus assay in mice (*in vivo*), a photo-mutagenicity assay in Chinese hamster ovary cells (chromosomal aberration test) and a photo-mutagenicity assay in E. coli (reverse mutation). In these all tests there was no evidence that drometrizole trisiloxane was genotoxic.

The validity of these tests could be questioned on the grounds that drometrizole trisiloxane (MW 500) may not have been able to penetrate cells to interact with genetic material. If penetration was based only on MW, then it is possible penetration occurred since a number of antibiotics are around this MW and others exceed it by up to 3-fold (vancomycin, MW 1449). However, cell penetration is based on more factors than just size and an exact understanding as to whether drometrizole trisiloxane penetrates into bacterial and mammalian cells (*in vitro* assays) appears unavailable.

Certainly, oral administration of drometrizole trisiloxane in the *in vivo* assay would have resulted in limited exposure even at a dose of 2000 mg/kg; it is worth noting that at this dose there was no

evidence of toxicity in mice. A dose of 2000 mg/kg, based on absorption data, would likely result in approximately 2 mg/kg drometrizole trisiloxane in plasma.

Carcinogenicity

The sponsor provided a comment (see attachment) by Dr G. Nohynek on the carcinogenicity potential of drometrizole trisiloxane. Dr Nohynek identified generally recognised criteria for the rationale to perform carcinogenicity studies on a substance as including large human systemic exposure to the substance, large population exposure, long duration of human exposure, structural alert features of the substance, genetic toxicology results indicating a mutagenic, clastogenic or other genotoxic potential, adverse findings in *in vivo* repeated-dose toxicity studies (immune modulation, hormonal modulation and non-neoplastic cell changes) and toxicokinetic studies showing formation of active metabolite/s (stored in tissues).

In the rationale for absence of a dermal carcinogenicity study these points were discussed with regard to drometrizole trisiloxane. It was noted that the likely systemic exposure would be low since the products containing drometrizole trisiloxane are for dermal application and drometrizole trisiloxane is poorly absorbed through the skin (0.32% in human skin *in vitro*). Therefore, the expected systemic of drometrizole trisiloxane is very low limiting the overall burden on the body.

The sponsor noted that UV filters are used seasonally, with their major use during the holiday season (except for rare case of professional use) and exposure of the general population is intermittent and chronic (daily long-term exposure). This statement is more likely associated with the European environment and not the Australian situation. In some parts (northern areas) of Australia sunscreens may need to be used daily all year round, while the higher UV levels measured across the entire Australian continent required extended (compared with Europe) use of sunscreens by the generally public.

The sponsor noted that chemical structural alerts for potential carcinogenicity and genotoxicity are well known and have been widely described in the literature (references cited and checked). Such chemical features have been applied as guidance criteria for the extent of required toxicology testing of food ingredients or fragrance substances. Reviewing structural features of drometrizole trisiloxane reveals no features that suggest potential carcinogenicity of mutagenicity activity of the molecule.

The sponsor noted that the genotoxic potential of drometrizole trisiloxane had been tested in a series of *in vitro* and *in vivo* assays (bacterial and mammalian cells), which had all been negative for mutagenic activity. Included in the assays examining genotoxicity potential were photo-mutagenicity assays using E.coli and CHO cells; these also produced negative results. It was noted that based on this information it extremely unlikely that drometrizole trisiloxane would be a carcinogen. It was noted that sensitisation potential may possess certain predictive value for carcinogenicity, since sensitising molecules are either electrophilic or form electrophilic metabolites that result in adducts to proteins and, potentially, to DNA. Therefore, the absence of discernible sensitisation potential of drometrizole trisiloxane confirms the non-reactive nature of the molecule to biological molecules.

The sponsor introduced further supporting analysis for a lack of carcinogenicity activity by including negative results from the repeated-dose subchronic, chronic and reproductive toxicity studies. There had been no evidence of non-neoplastic changes associated with drometrizole trisiloxane given by either the oral or dermal routes to rats or mice for 13 to 26 weeks. There were no indications of a potential target organ or effects on reproductive organs that may be suggestive of an interaction with hormonal activity.

The metabolism of drometrizole trisiloxane was investigated in vitro after incubation of rat liver microsomes and rat or human hepatocytes. No metabolites were found in the test. This result indicates there would be limited or no risk of the formation of an active metabolite.

Dr Nohynek concluded by highlighting the preceding points to re-state the belief that there was no evidence that triggers a concern about a potential carcinogenicity risk for drometrizole trisiloxane. He acknowledged that this view was shared by the SCCNFP, which rated the carcinogenicity potential of drometrizole trisiloxane as low and approved its use in sunscreens at concentrations up to 15%.

A second commentary by Dr R. Mascotto reasserted the rationale for the low carcinogenic risk of drometrizole trisiloxane. He reiterated that there was minimal human exposure to percutaneous drometrizole trisiloxane, an absence of alert features for the molecule, the molecule is stable, an absence of genotoxicity, an absence of sensitisation activity, an absence of photo-toxicity and photosensitisation, an absence of non-neoplastic changes in repeat-dose toxicity studies, an absence of adverse effects on reproductive function and hormonal balance, and an absence of metabolism (*in vitro*) that could lead to active metabolites.

These points are valid and in the context presented refer to likely systemic effects that may lead to carcinogenic activity. Dermal carcinogenicity is of equal concern (to systemic carcinogenicity) when considering topically applied products that will be used over extended periods of time. As mentioned above, negative results for skin sensitisation studies (humans and guinea pig) indicate negligible interaction of the test material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and, potentially, to DNA.

A non-genotoxic mechanism for carcinogenesis involving chronic skin irritation and inflammation could be relevant for topically applied substances such as UV filters in sunscreens. However, data are available from skin irritation studies (in rabbits, guinea pigs and humans), which indicate drometrizole trisiloxane was not a primary skin irritant.

In the only repeat-dose dermal toxicity study (range-finding) using mice, a formulation containing15% drometrizole trisiloxane was applied 5 days/week for 13 weeks without any significant adverse effects. There was a slightly increased incidence of epidermal hyperplasia in treated females compared with controls, but this response was not seen in males. Furthermore, with only one dose (15%) of drometrizole trisiloxane being administered there was no way to determine if this finding was part of a dose-related trend. The value of this study in providing supportive information for analysis of potential dermal carcinogenic activity is extremely limited due to its short duration, low number of animals (consistent with a dose-range finding study) and reduced dosing regimen.

Overall, important relevant properties of drometrizole trisiloxane that would suggest it is unlikely to possess potential dermal carcinogenic activity include; an absence of structural alert features in the molecule, photostability of the molecule, an absence of *in vivo* and *in vitro* genotoxicity, an absence of adverse activity at the skin (irritation, sensitisation, phototoxicity, photosensitisation) and an absence of metabolism of the molecule. The proposal that an absence of histopathological change in long-term oral and topical studies is supportive of no carcinogenic potential has limited application in this situation due primarily to extremely low systemic exposure following oral dosing and the relatively short duration of the studies.

ISSUES/RECOMMENDATION

The sponsor has submitted a package of data containing studies on drometrizole trisiloxane that conform to GLP standards and OECD or ICH guidelines. Drometrizole trisiloxane is a UV filter that has been on the accepted list of UV filters in the EU since 15/9/1998 at a concentration of up to 15% in products.

Studies submitted in support of this application cover all elements of the stated guidelines, except for specific interaction data, comprehensive repeat-dose (13 weeks) dermal toxicity data and a long-term dermal carcinogenicity bioassay (justification provided).

No definable toxicity in animal studies (repeat dose and reproductive toxicity, and genotoxicity) at 1000 mg/kg/day. Threshold dose for definable toxicity above 1000 mg/kg/day. Studies with animals and humans showed that drometrizole trisiloxane was not a skin irritant or sensitising agent, and it was not a phototoxic or photosensitising agent.

Toxicokinetic data indicated that systemic exposure following oral or dermal administration of drometrizole trisiloxane was very low (<1%). This impacts on the interpretation of oral toxicity studies and estimated safety margin indicated in SCCNFP report. Actual NOEL (>1000 mg/kg/day) needed for determination of safety margin, which currently cannot be determined accurately.

Systemic exposure (based on dose/body weight) to drometrizole trisiloxane in animal studies at 1000 mg/kg/day PO was likely to be similar or slightly above expected exposure in humans following normal use of a sunscreen with 15% drometrizole trisiloxane.

Justification for an absence of a dermal carcinogenicity bioassay was based on toxicity profile (absence of toxicity) and no structural alert features presence in the molecule. The likelihood of drometrizole trisiloxane being carcinogenic would be low to negligible based on the information provided.

Overall, drometrizole trisiloxane has been approved as a UV filter (at 15%) in Europe and has been in the market place there for approximately four and a half years. In the data provided drometrizole trisiloxane displayed a lack of definable toxicity in the studies presented at systemic exposure levels likely to be slightly above those anticipated to occur in humans following dermal exposure. On this basis drometrizole trisiloxane appears to be acceptable for use as an active ingredient in listed/registered sunscreen products at a concentration of up to 15%.

The advice/opinion of the committee is requested.

EVALUATION OF SUBMITTED TOXICITY DATA

TOXICOKINETIC/PHARMACOKINETIC DATA

Determination of G4375 in rabbit plasma samples collected in a teratology study; CIT; study no. 1947 ATP; G. Fabreguettes; 2000; GLP/QA-yes (ref. 44).

In this study plasma samples collected during the conduct of a teratology study (from in-life study ref. 41) in Chinchilla rabbits were analysed. Analysis for G4375 was by HPLC and was conducted on 80 samples. A description of the analytical method included tested for limits of detection, specificity, linearity, repeatability (intra and day to day) and stability of plasma extract over the test period.

It was noted that the specificity was satisfactory. The detector response was linear over a concentration range of 0.05-50.00 µg/mL and the intra-day and day-to-day repeatabilities were within designated limits. Recovery percentage values were within nominal limits. Plasma extract samples were stable for 24 hours at room temperature. Analysis of controls showed that the levels of G4375 were below the limit of quantification.

In the treated animals, exposure (AUC data) to G4375 increased over the dose range, but not in a dose proportionate manner. Cmax values were generally reached at 2-4 hours post-dosing. The following table presented plasma concentration and AUC data generated in this study. This data shows that measurable amounts of G4375 were absorbed following oral dosing and these levels increased (not dose-proportionally) with increasing dose.

G4375 plasma concentrations (µg /mL) and AUC values over a 4 hours period

Dose (mg/kg)	0.5 h	1 h	2 h	4 h	AUC (0.5-4h) (µg.h/mL)
100	0.928	0.918	1.03	1.29	10.9
300	1.29	1.28	1.46	1.66	15.4
1000	1.86	1.81	1.85	1.63	17.9

Determination of G4375 content in rat plasma; RCC; study no. 635220/Rhone Poulenc Chimie; J. Schreitmuller; 1997; GLP/QA-yes (ref. 13).

This study was comprised of two parts, one examining a sample of the test material (expiration date Sept. 1996) for stability and impurities and the second looking at plasma levels of G4375 in rats treated orally for a period of 13 weeks. Analysis of the test material was prompted by the fact that the sample used in the study had an expiration date of Sept. 1996 and had to re-analysed for purity and stability. The test material was valid at the time of the 13 weeks study, but by the time the stored plasma samples were analysed the material had passed its expiry date.

Initially, analysis of the parent compound (G4375) and impurities was conducted using HPLC and standard agents supplied by the sponsor. In the second part of the study, plasma samples collected from a 13 weeks oral toxicity study (no. 607408, ref. 14) in rats were analysed for G4375. Satellite groups (5/sex) were included in the rat study at the 0 and 1000 mg/kg/day dose levels, which were sampled at week 4 and week 13 at 2 hours post-dosing.

The parent substance in the sample of G4375 analysed was found to represent 99.18% of the material tested. It was stated that this shows G4375 would remain stable in the plasma samples up until the time they were tested. Analysis revealed the following contents profile: G4375 99.18%, G4374 0.01%, G4412 0.26%, G4411 0.47% and G4378 0.06%, while two additional known impurities (Tinuvin P and G4414) could not be detected. Total impurities amounted to 0.8% of the material tested.

There was no detectable evidence of G4375 in the control samples at either 4 or 13 weeks. Males had plasma levels of 1.112±0.682 and 0.824±0.333 μg/mL at weeks 4 and 13, respectively. Females had plasma levels of 1.158±0.205 and 1.290±0.168 μg/mL at weeks 4 and 13, respectively. Overall, there were no significant differences in plasma levels of G4375 between males and females or at the time points (4 and 13 weeks) for analysis. It would appear that there was no evidence that accumulation of the test material took place, with plasma levels at 4 and 13 weeks essentially the same in both sexes.

Determination of G4375 content in mice plasma; RCC; study no. 662005/L'Oreal; A. Burgener; 1997; GLP/QA-yes (ref. 17).

The aim of this study was to examine the potential systemic exposure to G4375 following 13 weeks (5 days/week application) dermal application of G4375 in mice (from in-life study, ref. 16). Animals (12/sex/group) were divided into two groups, with one group receiving placebo treatment and the other had G4375 (15% w/w solution) applied dermally. The test material was applied as a volume of 50 μL/mouse during the course of the study.

Data generated by analysis of plasma samples showed male mice with a value of 1864±416 ng/mL and female mice with a value of 1920±329 ng/mL. The combined value for both sexes was 1892 ng/mL, with no significant difference between the sexes.

^{14}C-G4375 ADME study: absorption, plasma pharmacokinetics, tissue distribution and excretion balance after single administration by cutaneous (dermal) and oral routes to rats; CIT; study no. 13394 PAR; T. Appleqvist; 2002 (ref. 54).

This study examined the fate of labelled-G4375 following its dermal and oral administration to rats. Groups (4) of hairless Sprague Dawley and normal rats were used in this study according to the following protocol. Group 1 (12 normal rats/sex) received 150 mg/kg G4375 by oral gavage; Group 2 (12 hairless rats/sex) received 150 mg/kg by topical application; Group 3 (5 normal rats/sex) received 150 mg/kg by oral gavage; Group 4 (5 hairless rats/sex) received 150 mg/kg by topical application. Groups 1 and 2 were used for plasma pharmacokinetics, while groups 3 and 4 were used for excretion balance testing. For groups 1 and 2, blood was collected (from 3

rats/sex/time point) at 0.5, 1, 2, 3, 4, 6, 8, 10, 24, 48, 72 and 96 hours. Groups 3 and 4 had urine, faeces and cage-wash collected pre-dose and then over regular periods up to 168 hours post-dosing periods. At the completion of the study the animals sacrificed and skin samples collected for analysis.

A dose-volume of 5 and 1 mL/kg was used for the oral and dermal routes, respectively. Vehicles for the oral and dermal routes were 4% aqueous methylcellulose/1% Tween 80 and Finsolv TN, respectively. During the study the animals were monitored for clinical signs of toxicity, morbidity and mortality. Body weights were measured at pre-dosing and at sacrifice.

During the conduct of the study there was no evidence of clinical signs of toxicity or deaths. Furthermore, due to the low recovery of material from the plasma assessment of potential metabolism could not be carried out.

Following oral dosing, the mean plasma levels of radioactivity rose rapidly to a Cmax of 430-484 ng.eqv/g (both sexes) at 0.5 to 1.0 hours before decreasing rapidly to levels below quantification (<322 ng.eqv/g) at the 2 hours analysis point. Following dermal application (8 hours exposure) the mean total radioactivity in plasma and blood were generally below quantification limits. There were instances where plasma levels of G4375 were measurable after dermal application, but these were infrequent and resulted in a determined maximum concentration of 280 ng.eqv/g. Due to limited data generated pharmacokinetic modelling and determination of exposure (AUC data) to G4375 were not possible.

It could be concluded that the bioavailability of G4375 after oral or topical/dermal dosing was very low.

Excretion balance data for the oral route of administration showed that both sexes had virtually identical handling of the labelled test material. Data collected over 168 hours revealed extremely low levels of radioactivity in the urine (0.04-0.05%), the bulk of radioactivity in the faeces (96.7-101.8%) and the remaining radioactivity in the cage wash sample (0.02-2.36%). Total recovery of radioactivity for the oral route was 99.1-101.8%.
Excretion balance data for the topical route of administration showed that both sexes had similar handling of the labelled test material. Data collected over 168 hours revealed very low levels of radioactivity in the urine (0.31-0.33%), with the majority of radioactivity in the faeces (46.0-53.2%), and small amounts of radioactivity in the cage wash sample (2.40-2.48%), application site on the skin (0.12-0.18%) and in the carcass (1.32-2.17%). Total recovery of radioactivity for the topical route was 73.0-82.5%. It was noted that there was low absolute total recovery of radioactivity for both sexes following topical dosing, which was unexplained apart from a suggestion that it may have been due to losses of radioactivity as $^{14}CO_2$. There was no evidence of selective storage of radiolabel during the course of the study.

Overall, very poor absorption was evident for both routes of administration, which indicates that there was very low bioavailability and minimal systemic exposure to G4375. The high radiolabel content in the faeces of the animals treated topically is unexpected since they wore protective collars during the treatment period and had the application site washed prior to removal of the

collar. An explanation could be inadequate washing of the application site and subsequent licking/grooming activity leading to significant ingestion of the labelled test material.

Using urinary radioactivity concentrations as an estimate of absorption leads to an assumption that <0.1% and <0.4% were absorbed following oral and dermal administration in the rat, respectively. If the animals did ingest some of the test material by licking the application (appears highly likely with radioactivity seen in faeces), then a small component of the estimate for absorption following dermal application may be due to oral absorption.

Etude de penetration in vitro sur humaine dermatomee; L'Oreal Dept. Recherche Appl.; study no. 94/04/26 et 94/04/28; F. Benech; 1994 (ref. 28).

Note: this was the format of presentation for this study. An English version of this article was submitted with the original French copy. The English title was:- G4375, study of penetration in vitro on dermatomed human skin.

A formulation (identified as 271.958:base 700 A0) containing 10% G4375 was applied to human skin samples and percutaneous penetration measured after 16 hours application. Analysis of the formulation plus active showed that G4375 was present at 10.550±0.087% w/w. Measurement of the skin surface (determined in skin wash) after completion of the exposure time indicated that 99.5±2.65% of G4375 was detected. The receptor fluid was found to contain <0.03% G4375 on analysis. A portion (<0.5%) of the test material must have remained in the dermatomed skin sample. Overall, the mean amounts of G4375 present after 16 hours application in the dermis and receptor-fluid compartments, which can be considered as the amounts having penetrated (or available for penetration), can be estimated at **<0.5%** of the dose applied.

In vitro percutaneous of Drometrizole Trisiloxane; L'Oreal Labs.; study no. 16004; F. Benech; 1998; GLP/QA-yes (ref. 29).

The purpose of this study was to examine the *in vitro* percutaneous absorption and cutaneous distribution of drometrizole trisiloxane (20%) formulated in an oil/water emulsion through human skin. The testing process involved the positioned of dermatomed human skin samples in static diffusion cells (n=12) followed by the application of the formulation containing 20% drometrizole trisiloxane. The contact period with the skin sample was 16 hours after which the skin was analysed for partitioning of the test material within the layers of the skin.

Results showed the applied dose to the skin surface was 101.71±3.22% of the intended dose. A breakdown of the distribution (as % of applied dose) of the test material showed the stratum corneum contained 0.80±0.18%, the epidermis contained 0.37±0.16%, the dermis contained 0.09±0.08% and the receptor fluid contained 0.23±0.04%. The total recovery amounted to 102.32±3.21%. It was noted that the absorbed amount of drometrizole trisiloxane remaining in the skin (stratum corneum, epidermis, dermis and receptor fluid) represented 1.49±0.30% of the applied dose. The amount that penetrated after 16 hours contact was represented by the combined values for the receptor-fluid and dermis, which totalled **0.32±0.10%** of the applied dose.

Pharmacokinetic study after single cutaneous application in mice of ^{14}C-G4375; CIT; study no. 13295 PAS; C. Fabreguettes; 1996; GLP/QA-yes (ref. 30).

In this study labelled G4375 was administered to a group (30 animals/sex) of Swiss mice as a single cutaneous application to aid the understanding of its pharmacokinetics. Each animal was treated (0.1 mL/animal) with a single dose of 47.5 mg G4375 as a 47.5% solution in acetone. The application site was not washed at any stage after treatment. Blood samples were taken from 5 animals/sex at 2, 4, 8, 24, 48 and 72 hours after dosing. Additional animals were included in this study to test the extent of G4375 removal by licking; these animals (2 animals/sex/sampling time) had protective dressings applied to the application site to prevent removal by licking. Testing for removal by licking took place at 2, 4 and 8 hour time points.

It was noted that there were no clinical signs of toxicity or unscheduled deaths during the study.

In animals without a protective dressing, values of Cmax (3.18-3.69 µg-eq/g), AUC (173-197 µg-eq.h/g) and C_{72h} (1.64-2.30 µg-eq/g) were similar in both sexes. However, the Tmax (h) was much longer in male (24.0) when compared with female (4.0) mice; no explanation was offered for this difference. An estimation of absorption using the data showed that less than 1% of the radioactivity was absorbed, regardless of sex.

In animals with a protective dressing, values for Cmax (0.124-0.189 µg-eq/g) and tmax (4-8 h) were presented. These results showed that the levels of absorption of radioactivity were significantly less in the animals with protective dressings compared to those without. Th comparison of data generated in this study showed animals without a dressing had a 20 to 26 times greater absorption of radioactivity than animal with a dressing. This is unusual since occlusion of topically applied substances generally leads to significantly increased absorption and not decreased absorption. This could be due to the observed high absorption (between 71.8% to 94.7%) of radioactivity unto the dressing, limiting the amount available for cutaneous absorption. It could also be partially due to oral absorption in animals without a dressing, following licking the site of application. Its worth noting that in general there were no significant differences between the sexes, except for tmax.

Pharmacokinetic study after single cutaneous application in rats of ^{14}C-G4375; CIT; study no. 13294 PAR; C. Fabreguettes; 1996; GLP/QA-yes (ref. 31).

In this study labelled G4375 was administered to a group (10 animals/sex) of hairless OFA hr/hr rats as a single cutaneous application (10% of total body surface) to aid the understanding of its pharmacokinetics. Each animal had a film as thin and uniform as possible of a 15% G4375 solution (in acetone) applied over an interscapular area, which administered an amount equivalent to a single dose of 75 mg G4375/kg. The quantity of the test material administered to each animal was adjusted to the body weight. The application site was covered with a dressing, which was left in place for an 8 hours period. Following the designated application period the dressing was removed and the site rinsed with water for injections. Dressings were kept for analysis to determine the amount of residual radioactivity retained.

Blood samples (1 mL) were taken from the same 5 animals/sex at 1, 4 and 24 hours after dosing, while the second 5 animals/sex had samples taken at 2, 8, 48 and 72 hours after dosing. This meant that each animal had 3-4 blood samples taken during the study. At the 48 hours time point plasma levels were above the limit of detection so additional blood samples were taken at 72 hours. Clinical signs of toxicity, mortality and body weights monitored during the course of the study.

It was noted that there were no clinical signs of toxicity or unscheduled deaths during the study.

Data showed values of Cmax (2.22-2.90 µg-eq/g), t_{max}(1.0-8.0h), $AUC_{(0\text{-}72h)}$ (89.9-102.1 µg-eq.h/g) and C_{72h} (0.75-1.14 µg-eq/g) were similar in both sexes. An estimate based on these data determined that the amount of administered dose found in the plasma was less than 1%. The pattern of results observed was similar to that seen for mice, with Cmax, $AUC_{(0\text{-}72h)}$ and C_{72h} all found to be similar for both sexes, while t_{max} displayed considerably variability between sexes. It was noted that a large quantity (82-93%) of radioactivity was found in the dressing. It was concluded that absorption of radioactivity (linked to labelled G4375) occurred after cutaneous application of a 15% G4375 solution in hairless rats. Uptake of radioactivity was low and elimination was slow.

Pharmacokinetic study in blood after single administration by oral (gavage) route in male rats of ^{14}C-G4375; CIT; study no. 11738 PAR; C. Fabreguettes; 1995; GLP/QA-yes (ref. 32).

The pharmacokinetics of orally (gavage, 5mL/kg) administered labelled G4375-(^{14}C), following a single dose of 1000 mg/kg to male Sprague Dawley rats, was examined through the measurement of plasma radioactivity. Also assessed in this study was the effect of two different vehicles (0.5% carboxymethylcellulose/Tween 80 and corn oil) on the extent of absorption of the radioactivity. Two groups each containing 9 male Sprague Dawley rats received labelled G4375 a single oral dose of 1000 mg/kg in either 0.5% carboxymethylcellulose/Tween 80 (group 1) or in corn oil (group 2). The general condition of the animals was followed over the course of the study. Blood samples (1 mL) were taken from 3 animals at 30 minutes, 1, 2, 4, 8, 24, 48 and 71 hours post-dosing, as well as at 139 hours in the corn oil group only. Each animal gave 2-3 blood samples spread over the sampling period to lessen the physical impact/trauma. Clinical signs of toxicity, mortality and body weights were monitored during the course of the study.

It was noted that there were no clinical signs of toxicity or unscheduled deaths during the study.

It was noted that there was measurable radioactivity in plasma after a single oral dose of G4375 (1000 mg/kg) in male SD rats, which was seen regardless of the vehicle used. Absorption was more rapid in aqueous 0.5% carboxymethylcellulose/Tween 80 than in corn oil as indicated by the tmax values (2 vs 8h, peak conc. 2.08 vs 1.63µg-eq/g). The rate of elimination was also more rapid for G4375 in 0.5% carboxymethylcellulose/Tween 80 compared with corn oil (elimination t1/2 13.9 vs 37.3h). The Cmax was slightly higher when 0.5% carboxymethylcellulose/Tween 80 was the vehicle (2.08 vs 1.63µg-eq/g), but the total exposure (AUC µg-eq.h/g, 63.3 vs 46.3) and volume of distribution (Vd/F L/kg, 605 vs 384) were greater when corn oil was the vehicle.

Stability study of G4375 in rat and human plasma; ADME Bioanalyses SA; study no. ORE/G4375/94001/L'Oreal; P.Y. Communal; 1994 (ref. 33).

This study examined the stability of G4375 in human and rat plasma using two methods, one validated in the range of 100-1000 ng/mL and the second over the range of 10-200 ng/mL. These methods involved the use of liquid-liquid extraction followed by specific HPLC analysis using a C18 column and UV detection. The stability of G4375 was studied for 24 hours at 37^0C over the following range of concentrations, 20, 50, 100, 200, 500 and 1000 ng/mL in rat plasma and at 1000 ng/mL in human plasma and deionised water.

At the validation phase, data on calibration curves, intra-run precision and accuracy (repeatability), inter-run precision and accuracy (reproductibility), limit of quantification, recovery and specificity were presented for both methods and concentration ranges. Rat plasma was collected from fasted male Sprague Dawley rat, which were sacrificed by exsanguination. Human plasma was obtained frozen from the Centre de Transfusion Sanguine (Lyon, France).

In human and rat plasma, results indicated that at 1000 ng/mL G4375 concentration changes measured at time points up to 24 hours did not vary significantly and were not time-related. All values remained above the nominal concentration of 1000 ng/mL. In rat plasma at concentrations of 20-500 ng/mL, concentration changes measured at time points up to 24 hours did not vary significantly and were not time-related. G4375 was stable in human and rat plasma at concentrations up to 1000 ng/mL over a 24 hours period.

In vitro metabolism of G4375 by rat hepatocytes and by hepatic rat and human microsomes; ADME Bioanalyses SA; study no. ORE/G4375/94002/L'oreal; B. Neau; 1994; GLP/QA-yes (ref. 34).

Steps described for the conduct of this study were measurement of the viability of rat hepatocytes, preparations of solutions of G4375 and incubation of G4375 with the hepatocytes. Similarly for the rat and human microsomes, control of liver microsomes by measurement of cytochrome P-450 content, preparations of G4375 solution and incubation of microsomes with G4375 and ^{14}C-testosterone. Sources of microsomes to be used in the study were characterised for humans (3 caucasian males) as HOM7, HOM10 and HOM26 and for rats (1/sex Sprague Dawley) as SDM and SDF. Microsomal viability was checked using the method of Omura and Sato (1964). The NADPH regenerating system was tested with each microsomal species by measuring the hydroxylation of ^{14}C-testosterone (Laroque et al, 1988). Stock solutions (10^{-2} or 10^{-3}M) of G4375 were prepared in pure DMSO. Dilution to the required concentrations was achieved using buffer solutions (William's E buffer). The incubation times employed were 1, 3, 6 and 24 hours for each concentration tested in duplicate.

Viability (morphological assessment and erythrosine exclusion test) and attachment efficiency of hepatocytes after isolation were seen to be in agreement with published data. Examination (LDH leakage, morphology, erythrosine exclusion) of the cultured hepatocytes revealed they were quite similar to histologic preparations from normal animals. Measurement of cytotoxicity of G4375 at the concentrations tested (10^{-4} to 10^{-7} M) was negative indicating no adverse effect of G4375 on cell viability.

In vitro metabolism of G4375 by rat hepatocytes and by rat and human microsomes produced results showing an area of the G4375 peak, which corresponded to about 80% of the peak observed for the blanks. No other peaks, besides those observed in the blank chromatogram or for G4375 itself, were observed. In the control test of ^{14}C-testosterone, incubation resulted in production of major hydroxylated metabolites, which was stated to validate all the microsomes used in the study. Results showed that approximately 80% of the G4375 in the initial incubation mix were recovered unchanged. An explanation for the reduced recovery rate (80%) of G4375 was the presence of minor metabolites that were not detected under the HPLC procedure employed or due to loss of G4375 during the analytical procedure.

LOCAL TOLERANCE AND PHOTOTOXICITY

Primary eye irritation study with G4375 in rabbits; Research & Consulting Company; Study No. 607386; W. Braun, 1996; GLP/QA-yes (ref. 5).

The primary ocular irritation potential of G4375 was examined by instilling 0.1 g into one eye of each of 3 NZ white rabbits (1 male, 2 female). The treated eyes were not rinsed and the untreated eyes acted as a control. Irritation was scored at 1, 24, 48 and 72 hours after application of the test material. Cumulative score assessment was manipulated statistically to generate a mean irritation rating. The primary irritation score was calculated by totalling the individual cumulative scores at 24, 48 and 72 hours and them dividing by the number of criteria/observations.

Staining of the tissues surrounding the eye failed to show any uptake of dye by the cornea, sclera or conjunctivae. This indicated that these tissues did not suffer any disruptive damage following application of the test material. A slight degree of conjunctival redness was observed in each of the treated eyes and slight chemosis was observed in 2/3 eyes.

The result of the calculation described above was a primary ocular irritation score of 1.11 (max. possible 13). This result indicated that the test material was classified as a not irritating agent to the rabbit eye (according to EEC Commission Directive 93/21/EEC). The slight irritation observed may have been due to direct irritation of a solid (0.1 g) placed in the eye and not to ocular reactivity of the agent.

Primary skin irritation study with G4375 in rabbits – 4 hours semi-occlusive application; RCC Labs. Ltd., Switerland; Study No. 607375; W. Braun; 1996; GLP/QA-yes (ref. 4).

The primary skin irritation potential of G4375 was examined following the topical (dermal) application of 500 mg onto clipped intact dorsal skin (6 cm^2) of NZ rabbits (1 male, 2 female). The treated site was covered with a surgical gauze patch, which was in turn covered by a semi-occlusive dressing. The dressing was held in place for 4 hours and following its removal the treated site was washed clean with lukewarm tap water. Scoring of skin reactions took place at 1, 24, 48 and 72 hours after removal of the dressing. Tabulation of the mean scores was based on discernible skin reactions at 24, 48 and 72 hours after removal of the dressing.

The data indicated that G4375 had a primary irritation score of zero, with no evidence of erythema or oedema at any of the evaluation times. No corrosive effects were noted on the treated skin of any of the 3 animals at any evaluation time (including at 1 hour). Overall, G4375 was found not to be a primary skin irritant in the rabbit.

Sensitisation study in the guinea pig – Magnusson/Kligman assay; Toxicol Labs. Ltd.; Study No. A/K38711; H. Teale; 1993; GLP/QA-yes (ref. 6).

This study was performed to assess the delayed hypersensitivity response of G4375 in the guinea pig. A range-finding preliminary study was conducted to determine the appropriate doses of the test material to use in the main study. For induction, an intradermal injection dose of 0.1 mL at 25% was found to be non-irritating, while the highest concentration (80%) tested topically was found to be non-irritating. Topical induction was achieved using 4 cm^2 pieces of filter paper saturated in the test material applied to the test site. G4375 batch RF004.

In the main study, a total of 30 female Dunkin Hartley albino guinea pigs were used, with 10 allocated to a control group and 20 the test group. For the induction phase of the main study, doses found suitable were 25% (in corn oil) for intradermal (ID) administration and 80% (in corn oil/acetone) for topical application (two phase induction using ID and topical routes). Included in the induction phase was Freund's Complete Adjuvant as per the requirements of the Kligman maximisation test. The timing of treatment for the induction consisted of an initial ID injection of the test material followed 7 days later by topical application of the test material on filter paper, which was held in place for 48 hours under an occlusive dressing.

A total of 14 days after the induction the challenge treatment involving the topical application of 60 % and 80% solution (in corn oil/acetone) was initiated, with the application site covered with an occlusive dressing for 24 hours. The test sites were examined for skin reaction at 24 and 48 hours after removal of the challenge patch (filter paper).

Results were based on the Magnusson and Kligman classification system shown below:

Sensitisation rate (%)	Grade	Classification
0-8%	1	weak
9-28%	2	mild
29-64%	3	moderate
65-80%	4	strong
81-100%	5	extreme

In the main study, one of the 20 test animals produced a positive response, which represents a response rate of 5% and places the test material as a weak sensitiser with a grade 1 classification. The challenge phase involved the application of two concentrations (60% and 80%) of the test material at different sites, which assessed at 24 and 48 hours post-challenge. The responding animal displayed a low-grade 1 response described as scattered mild redness only at the 24 hour inspection and only at the 80% concentration of the test material.

It should be noted that this study used the Kligman maximisation method, which sensitises the responsiveness of the test system.

Contact hypersensitivity study in the guinea pig – Maximisation assay; RCC Labs. Ltd., Switerland; Study No. 607397; G. Arcelin; 1996; GLP/QA-yes (ref. 7).

In a second sensitisation study, 20 male Ibm:GOHI; SPF male guinea pigs (Himalayan spotted) were induced intradermally (ID) once a week in the first week and topical once a week in the second week with G4275 at 5% (0.1 mL) and 85% (8 cm^2 saturated filter paper) in corn oil/acetone, respectively. A vehicle control group had 10 animals. Appropriate doses of the test material had been determined in a dose range-finding study. Included in the induction phase was Freund's Complete Adjuvant as per the requirements of the Kligman maximisation test. G4375 batch 95003/B.

At 2 weeks after the induction process the animals were challenged with the highest concentration of 85% G4375 (4 cm^2 saturated filter paper applied topically) used in the induction phase; the challenge patch was left in place for 24 hours.

Challenge application sites were assessed for skin reactions approximately 24 and 48 hours after removal of the challenge patch. Scoring of the skin reactions was carried out according to the Draize system.

In general, no erythematous or oedematous reaction was observed in animals treated with the corn oil/acetone alone or with the test material (85%). There were no deaths during the course of the study. There were no clinical signs of toxicity observed in any animals during the course of the study. Body weights of test and control animals were similar over the course of the study.

Results indicated that none of the control or test animals displayed any evidence of a positive response to the challenge application of the test material. The test material was determined to be a non-sensitiser in this assay.

Contact hypersensitivity study in the guinea pig – Modified Buehler assay; RCC Labs. Ltd., Switzerland; Study No. 610784; G. Arcelin; 1996; GLP/QA-yes (ref. 8).

In this study 20 females of the test group were treated topically with G4375 at 85% in corn oil/acetone and 10 control group females were treated with corn oil once a week for a 3 weeks induction phase. The patching method was used for irritation screening, induction and challenge. Patching was achieved using the Hill Top Chamber, which was saturated with approximately 0.1 g of the test material in corn oil/acetone. The chamber was applied to a shaved area of the left shoulder and held in position for 6 hours by an occlusive dressing. After the last induction exposure the animals were left untreated for 2 weeks. G4375 batch 95003/B

At 2 weeks after the end of the induction period both groups were challenged with G4375 at the same concentration used during the induction. Irritation screening for the induction and challenge phases was conducted on 4 animals. A positive control sensitivity validation (α-

hexylcinnamaldehyde) had previously (1995, according to OECD guidelines) been used to validate the procedure/method; this was not a concurrent assay. Method validation had been supported by a 58% positive reaction rate for the positive control agent in assays using the same protocol.

In the irritancy screening section of the study, there was no evidence of irritation at concentrations of test material (in corn oil/acetone) up to the highest (85%) level used. In the main study, there was no evidence of skin reactions during induction or at challenge in either the test or control group. There were no deaths or clinical signs of toxicity during the course of the study. Body weights of test and control animals were unaffected during the course of the study. It was concluded that G4375 was not a skin sensitiser.

Two weeks skin tolerance study with G4375 on the albino guinea pig; RCC Labs. Ltd., Switzerland; Study No. 607151; G. Arcelin; 1996; GLP/QA-yes (ref. 11).

In this study, the test material (0.04 g of 85% in corn oil/acetone) and vehicle (0.1 mL/2 cm^2) were applied on the right and left flank of 6 (3/sex) Ibm:GOHI; SPF guinea pigs (Himalayan spotted), respectively, for 14 consecutive days. The application sites were left uncovered during the course of the study. G4375 batch no. 95003/B.

There were no deaths or clinical signs of toxicity during the course of the study. Body weight change was similar in the treatment and control groups. Inspection of test and control application sites on the flanks of the animals noted that there were no adverse changes detectable. Microscopic examination of treated tissue (to depth of 4 μm) indicated no abnormal incidence of change to the skin. Overall, the topical application of at a concentration of 85% in corn oil/acetone daily for 14 days had no adverse effect on shaved skin of the guinea pig.

Test to evaluate photoallergic potential in the guinea pig; Pharmakon Europe; Study No. 15794; O. Mercier; 1994; GLP/QA-yes (ref. 9).

The photoallergic potential of G4375 was assessed in albino Hartley guinea pigs (30 animals, 15/sex) using three groups in the main study. A preliminary study was conducted using 4 animals (2/sex) to determine an appropriate non-irritating dose of G4375, which was found to be the highest used (60% w/v). There was no evidence of irritation at 24 or 48 hours after exposure of either 30% or 60% G4375 in the presence or absence of irradiation. Groups making up the main study were the test (5/sex receiving G4375) group, and positive (5/sex receiving 2% tribromosalicylanilide) and negative (5/sex receiving the vehicle) control groups. The vehicle consisted of dimethylacetamide, acetone and ethanol (40:30:30). The test group used 0.1 mL of G4375 at a concentration of 60% (w/v) in the vehicle, which was the maximum non-irritant concentration. Application sites (2 cm x 2 cm) were on the dorsal and flank regions of the animals and were cleared of fur using depilation cream. Application was followed by irradiation with a sub-erythematogenic dose level of UVA only. G4375 batch RF 004.

Induction: The animals were sensitised with 4 intradermal (ID) injections of Freund Complete adjuvant (used in maximisation assay). A volume of 0.1 mL G4375 (60%) was applied topically for 30 minutes, which was followed 30 minutes later by irradiation with a sub-erythematogenic

dose level of UVA and UVB. Topically treatment with G4375 and irradiation was repeated 5 times over a 2 weeks period. Both positive and vehicle control groups followed this procedure.

Challenge: Topically application of G4375 for 30 minutes was followed by irradiation with a sub-erythematogenic dose level of UVA only. Cutaneous macroscopic evaluation was conducted using the Draize scale at 24 and 48 hours after the end of the irradiation. Both control groups underwent similar procedures.

There was no evidence of adverse effects on the skin as a result of the treatment described above. All 10 animals receiving the G4375 were free of skin irritation (erythema or oedema) at the induction and challenge sites, while all 10 animals in the positive control group displayed erythema at the irradiated sites but not the non-irradiated sites. The vehicle control group did not show any sign of skin irritation. Overall, G4375 did not generate any phototoxic or photoallergic reactions in guinea pigs in a hypersensitivity study.

Determination of photoallergicity with G4375 in albino guinea pig; Research & Consulting Company; Study No. 607162; O. G. Arcelin; 1996; GLP/QA-yes (ref. 10).

This study examined the photoallergenicity potential of G4375 (batch 95003/B) in Ibm:GOHI; SPF male guinea pigs (Himalayan spotted). A total of 30 animals were used in the main study and 4 animals were used in a preliminary dose-ranging study. In the pre-test the highest non-irritating concentration used (85% G4375 in acetone) was found to be a non-irritating level, which was used in the main study for both the induction and challenge phases. In the main study, a group of 20 animals underwent induction with an initial intradermal (0.1 mL) dose of Freund Complete adjuvant, which was followed by topically applied G4375 (85% in acetone) administered to an area of 8 cm^2 on 5 occasions over 2 weeks period. It was noted that the test article was applied to cover the entire test article site, but the exact amount applied was not determined. The test site was exposed to 1.8 J/cm^2 UVB and 10 J/cm^2 UVA irradiation.

There was no mention of a positive control group being run concurrently in this study. There was mention of positive (using tetrachlorosalicylanilide) control data generated from the laboratory, but the timing of this study was unclear. The positive control did elicit an expected response in the reference data provided in the guinea pig (strain not described). This historical data was meant to show that the guinea pig is an appropriate model for the investigation of photosensitisation reactions.

Challenge was initiated 3 weeks after induction, with animals administered the highest non-irritating concentration (85%) of G4375. Both flanks (test sites) of the animals were exposed to this concentration, with one side irradiated (UVA only at 10 J/cm^2) and the other side not irradiated. Application of the test material was to a freshly shaved area of 2 cm^2, with the test substance (amount not measured) covering the entire site. Animals of the control group (10 started, but one died leaving 9 at assessment) were induced intradermally with Freund Complete adjuvant and treated at challenge with G4375 (85% in acetone).

In the initial preliminary concentration-ranging study, there was no sign of irritation or photo-irritation was evident at any concentration (60%, 70%, 80% and 85%) of G4375 tested. The data

from the main study showed that there was no evidence of erythema or oedema at the challenge sites (either irradiated or non-irradiated) in test and control groups at the 24, 48 or 72 hour assessments. One animal from the control was found dead on day 22 of the study, with an autopsy revealing reddish discolouration of the lungs. This death was considered unrelated to the test material. There was no evidence of clinical signs of toxicity or systemic toxicity in surviving animals. Body weights of test animals were slightly below the control animals at the end of the study. Overall, no positive reactions were observed at irradiated and non-irradiated sites of test and control animals after exposure to G4375 (85% in acetone) at challenge.

ACUTE TOXICITY

Acute oral toxicity; Department Central Securite Products, L'Oreal; Study No. 93/06/261; I. Delabarre & M. Cottin; 1993 (ref. 1).

This study was described as following very close to the fixed-dose (limit test) protocol recommended as an alternative to the LD50 by the British Toxicology Society. There was no statement of compliance to GLP principles or quality control/audit procedures.

This study examined the possible adverse effects following oral administration of G4375 to Charles River rats at an amount of 10 mL/kg in peanut oil (vehicle), which equated to a dose of 2 g G4375 in peanut oil/kg. A total of 6 animals (3/sex) were given a single oral dose by gavage, which was followed by a 14 days observation period. At the end of the observation period the animals were subjected to an autopsy.

There were no deaths during the dosing and observation periods. Results of the autopsy revealed very slight gastritis affecting the cardiac region of the stomach in all 3 males. There was evidence of necrosis to the liver with adhesions to the right kidney in 1 male; females were free of adverse findings. It was concluded that there was no acute toxic risk at a dose of 2 g/kg in rats.

Acute oral toxicity study with G4375 in rats; Research & Consulting Company; Study No. 607353; G. Arcelin, 1996; GLP/QA-yes (ref. 2).

The test agent G4375 was administered to a group of Wistar SPF rats (5/sex) by oral gavage at a single dose of 2 g/kg. G4375 was prepared for administration (in a volume of 10 mL/kg) by adding it to a vehicle comprising methylcellulose (4%) and Tween 80 (1%). The test material was suspended in the vehicle at a concentration of 0.2 g/mL. Animals were monitored for mortality, clinical signs of toxicity and body weight change over a period of 14 days prior to autopsy.

Results showed that all animals gained weight during the observation period. There were no clinical signs of toxicity during the observation period. There were no unscheduled deaths during the course of the study. At autopsy, there were no adverse findings for organs and tissues examined. Overall, there was no evidence of toxicity and the LD50 was determined to be greater than 2 g/kg.

Acute dermal toxicity study with G4375 in rats; RCC Labs. Ltd., Switzerland; Study No. 607364; G. Arcelin; 1996; GLP/QA-yes (ref. 3).

G4375 (batch 95003/B) was applied topically (dermal) to a group (5/sex) of HanIbm: Wistar (SPF) rats at a single dose of 2 g/kg (diluted in acetone). The test material was administered at a volume of 4 mL/kg to a clipped area approximating 10% of the total body area. After the dermal administration of G4375 the application site was covered with a semi-occlusive dressing. Treatment was followed by a 14 days observation period.

No deaths occurred during the study. There was no evidence of clinical signs of toxicity or local damage to the application site following administration of the test material. Body weight gain of all animals was within a normal range for the strain and species of rat used in the study. It was determined that the dermal LD50 for G4375 was > 2g/kg.

Acute dermal toxicity study in mice using G4375; RCC Labs. Ltd., Switerland; Study No. 753175; E. Rosner; 2000; GLP/QA-yes (ref. 50).

G4375 (batch 95004B) was applied topically (dermal) to a group (5/sex) of HanIbm: NMRI (SPF) mice at a single dose of 2 g/kg (diluted in 4% methylcellulose and 1% TWEEN). The test material was at a concentration of 0.33 g/mL and administered at a volume of 6 mL/kg to a clipped area approximating 10% of the total body area. After the dermal administration of G4375 the application site was covered with a semi-occlusive dressing. Treatment was followed by a 15 days observation period.

No deaths occurred during the study. There was no evidence of clinical signs of toxicity or local damage to the application site following administration of the test material. Body weight gain of all animals was within a normal range for the strain and species of rat used in the study. There were no abnormal findings observed at autopsy. It was determined that the dermal LD50 for G4375 was > 2g/kg.

Acute oral toxicity in mice; Research & Consulting Company; Study No. 753164; E. Rosner, 2000; GLP/QA-yes (ref. 49).

The test agent G4375 was administered to a group of HanIbm: NMRI (SPF) mice (3/sex) by oral gavage at a single dose of 2 g/kg. G4375 was prepared for administration (in a volume of 10 mL/kg) by adding it to a vehicle comprising methylcellulose (4%) and Tween 80 (1%). The test material was suspended in the vehicle at a concentration of 0.2 g/mL. Animals were monitored for mortality, clinical signs of toxicity and body weight change over a period of 14 days prior to autopsy.

Results showed that all animals gained weight during the observation period. There were no clinical signs of toxicity during the observation period. There were no unscheduled deaths during the course of the study. At autopsy, there were no adverse findings for organs and tissues examined. Overall, there was no evidence of toxicity and the LD50 was determined to be greater than 2 g/kg.

Acute intraperitoneal toxicity in rats; Research & Consulting Company; Study No. 753197; E. Rosner, 2000; GLP/QA-yes (ref. 47).

The test agent G4375 was administered once to groups of HanIbm: Wistar SPF rats (5/sex) by intraperitoneal (IP) injection at dose levels of 500, 1000, 1500 or 2000 mg/kg. G4375 was prepared for administration (in a volume of 12 mL/kg) by adding it to a vehicle comprising methylcellulose (4%) and Tween 80 (1%). Animals were monitored for mortality, clinical signs of toxicity and body weight change over a period of 14 days prior to autopsy.

Mortalities were observed at all doses in females (40% at 500 mg/kg, 80% at all other doses), but only at the highest doses in males (40% at 2000 mg/kg). All animals that died spontaneously died on test days 5, 6 or 7. Clinical signs of toxicity included ruffled fur, hunched posture and emaciation, bradypnea and ventral positioning. The 3 females that survived from the 500 mg/kg group showed reduced body weights one week after administration of the test material. However, by the end of the observation period the body weights had recovered to the normal range. At autopsy, adhesion of the liver lobes and/or adhesion of the intestines with the liver and the skin were observed at all dose levels. Fluid in the thoracic cavity or in body cavities was evident in several animals at all doses except 1000 mg/kg, while 2 females treated with 1000 mg/kg displayed reddish discolouration of the lungs shortly after death.

Overall, estimation of LD50 produced values of 563.87 mg/kg in females and greater than 2000 mg/kg in males. There is no obvious reason for this difference in acute toxicity between males and females.

Acute intraperitoneal toxicity in mice; Research & Consulting Company; Study No. 753186; E. Rosner, 2000; GLP/QA-yes (ref. 46).

The test agent G4375 was administered once to groups of HanIbm: NMRI (SPF) mice (5/sex) by intraperitoneal (IP) injection at dose levels of 500, 1000, 1500 or 2000 mg/kg. G4375 was prepared for administration (in a volume of 12 mL/kg) by adding it to a vehicle comprising methylcellulose (4%) and Tween 80 (1%). Animals were monitored for mortality, clinical signs of toxicity and body weight change over a period of 14 days prior to autopsy.

Mortalities were observed at the 3 higher doses in females (0% at 500 mg/kg, 40% at 1000 mg/kg, 100% at 1500 mg/kg and 40% at 2000 mg/kg), but only at the highest doses in males (20% at 2000 mg/kg). All animals that died spontaneously died on test days 5, 6 or 7. Clinical signs of toxicity included ruffled fur, hunched posture and emaciation and bradypnea. The majority of surviving animals showed reduced body weights one week after administration of the test material. However, by the end of the observation period the body weights had recovered to the normal range. At autopsy, adhesion of the liver lobes and/or adhesion of the intestines with the liver and the skin were observed at a dose 1000 mg/kg and above. Fluid in the thoracic cavity or in body cavities was evident in two females treated with 2000 mg/kg, while one females treated with 1000 mg/kg displayed reddish discolouration of the lungs shortly after spontaneous death occurred. A female given 500 mg/kg displayed pale discolouration of the kidneys at scheduled necropsy.

Overall, estimation of LD50 produced values of 1200.94 mg/kg in females and greater than 2000 mg/kg in males. There is no obvious reason for this difference in acute toxicity between males and females.

REPEAT-DOSE TOXICITY

Repeated-dose 14 days oral toxicity in rats; Research & Consulting Company; Study No. 365534; A. Dotti, 1995; GLP/QA-yes (ref. 12).

In this study G4375 was administered daily to SPF Sprague Dawley rat by gavage at dosages of 0, 100, 300 or 1000 mg/kg/day for a period of 14 days. Groups consisted of 5 animals per sex for each dose level. The vehicle for the test material was carboxymethylcellulose (2%) and Tween 80 (1 drop), with the test material delivered by gavage. The dose volume was 10 mL/kg. It was noted that the sponsor considered that absorption by the oral route would be at least equal to the percutaneous absorption possible following dermal application (proposed site for humans). Parameters followed during the course of this study included clinical signs of toxicity, food consumption, body weights, haematology, clinical biochemistry, organ weight analysis and microscopic and macroscopic pathology.

There were no unscheduled deaths during the course of the study and no evidence of clinical signs of toxicity in either the treated animals or controls. There were no changes in food consumption figures following a comparison of the treated and control groups. Body weight gain over the course of the study was similar for the treated and control groups. Haematological and clinical biochemistry parameters were found to be within a range of values that would be considered normal. Any fluctuation in these values was considered to be incidental or unrelated to the treatment and within normal biological variation.

Organ weight analysis was unremarkable, with changes attributed to biological variation and not treatment-related. Macroscopic findings (reddish/black discolouration or foci in organs and uterine horn) were minimal and occurred with similar frequency and intensity across all study groups (including controls). There were no microscopic findings that could be attributed to treatment with the test material. Observed changes were of a spontaneous nature and equally distributed across all groups. There was no evidence of any treatment-related adverse effect associated with use of the test material.

Overall, oral administration of G4375 at a dose up to 1000 mg/kg/day over 14 days had no obvious adverse effect on the rat (NEL 1000 mg/kg/day).

SUBCHRONIC TOXICITY

Subchronic 13-weeks oral toxicity (gavage) study with G4375 in rats; Research & Consulting Company; Study No. 607408; H. Schmid et al, 1996; GLP/QA-yes (ref. 14).

The test material (G4375, batch 95003/B) was administered to groups (5/sex/dose) of Sprague Dawley (SD) IcoIbm:OFA (SPF) rats for 13 weeks at dose levels of 0, 100, 300 or 1000 mg/kg/day PO. An additional 5 animals/sex/group for the 0 and 1000 mg/kg dose levels were

kept drug free for a further 4 weeks to determine likely recovery from any adverse effects. Also, a further 5 animals/sex/group for the 0 and 1000 mg/kg dose levels were used to obtain plasma samples at 6 and 13 weeks of treatment for the determination of clinical chemistry and haematology. Urine samples were also collected.

There were no treatment-related unscheduled deaths or clinical signs of toxicity during the course of the study. A control male was found dead on study day 35. Clinical signs seen at a similar frequency across all groups included alopecia, skin crusts, scar tissue on the skin, reddish sore spots and scabbed wounds. Overall, food consumption (and relative food consumption) and body weight gain was consistent across all groups. However, males from groups receiving 100 and 1000 mg/kg G4375 showed a slightly greater (5% to 6%) food intake from days 1 to 8 when compared to controls. There were no statistically significant increases in body weight for any treatment group when compared to controls.

Ophthalmoscopic examination was unremarkable for all groups, with a low but increased incidence (1/10) of corneal opacity in the high dose males compared with the controls (0/10) and high dose females (0/10). Organ weight analysis, gross pathology and histopathology were unremarkable with any changes identified as spontaneous in nature and within the normal range of background incidence rates for SD rats. There were no treatment-related detected in haematology and urinalysis parameters.

There were changes noted in clinical chemistry parameters. These changes are summarised in the following table.

Changes in clinical chemistry parameters

Parameter	% change from control	Sex affected	Time of change
Plasma glucose concentration	↓12%	Females (HD)	13 weeks
Total cholesterol level	↑21%	Males (HD)	6 weeks
	↑23%	Males (HD)	13 weeks
Creatine kinase activity	↑23%	Males (HD)	6 weeks
Plasma sodium concentration	↑3%	Males (MD)	6 weeks
Plasma protein fractions			
α1-globulin fraction	↑6%	Females (HD)	6 weeks
α2-globulin fraction	↓20-31%	Both sexes (HD)	6 weeks
	↓11%	Males (LD)	13 weeks
β-globulin fraction	↑8-10%	Both sexes (HD)	6 & 13 weeks
gamma-globulin fraction	↓29%	Females (HD)	6 weeks

Note: In the above table abbreviations include; LD - low dose (100 mg/kg/day), MD - mid dose (300 mg/kg/day) and HD - high dose (1000 mg/kg/day).

It was noted that none of these changes were evident after the 4 weeks recovery period, indicating their reversibility. In a discussion, it was stated that these changes were minor in degree and suggestive of metabolic adaptations; it was concluded that they were of no toxicological relevance.

Overall, the occurrence of dose-related trends for the majority of these parameters were not evident, with some effects only seen at the LD or MD, but not the HD. Also, a number of these effects were seen only in one sex. Increases (23%↑) in CK activity may have reached statistical significance, but changes in magnitude below a doubling are considered to be of no biological consequence. Furthermore, CK activity was found to have changed only in one sex and at one time point (6 weeks).

A decrease in glucose levels was seen in HD females at 13 weeks, while at 13 weeks the glucose levels were similar in the male HD group and controls. It is also worth noting that the direct opposite occurred at the LD and MD in males with increased glucose levels observed at 13 weeks.

Sub-chronic dermal toxicity with G4375: Preliminary tolerance study by dermal administration to CD-1 mice for 13 weeks; Huntingdon Life Sciences, study no. LOL/010/L'Oreal; I. Nicholls; 1997; GLP/QA-yes (ref. 16).

A group (12/sex) of CD-1 mice had a formulation containing G4375 (batch no. DEF/C 95001B), at a concentration of 15%, applied dermally (50 μL/mouse) at a frequency of 5 days/week for a period of 13 weeks. A control group (12/sex) received a vehicle formulation using the same timing as the test group. Application sites (dorsum) were clipped free of fur prior to application

of the test and vehicle formulations. The sponsor supplied pre-formulated G4375 in tubes (15%) and tubes of vehicle formulation were also supplied. Clinical signs of toxicity were followed twice daily, as well as examination of the application sites for irritancy. Parameters followed during the course of the study included body weights, food consumption and conversion efficiency, and macroscopic and histopathology.

This study included a statement that blood samples (after 13 weeks treatment) were taken to measure the extent of dermal absorption of the test material. However, the results from this part of the study were not included in this report, but were to be reported separately by the sponsor.

All aspects of chemistry and stability of the test and vehicle formulations were checked prior to use in the study and at the completion of the study. The vehicle formulation contained glycerol (5 g), triethanolamine (0.75 g), demineralised water (71.5 g), cetyl alcohol (0.5 g), Dow Corning 200 fluid (0.5 g), methyl ethyl propyl butyl isobutyl hydroxy benzoate phenoxy ethanol mixture (1 g), palm oil stearic acid (2.75 g), Arlacel 165 (1.5 g), C12-15 alkyl benzoate (15 g), potassium cetyl phosphate (1 g) and polyacrylamide (0.3 g). The test material contained the above ingredients and 15% G4375.

There were no deaths or clinical signs of toxicity during the course of the study. Bodyweight gains were slightly higher in the test group males when compared with controls, while food consumption was unaffected in the test group. Interestingly, the efficiency of food conversion for the test group males was higher than that of the controls.

Macroscopic and microscopic examination of the test and control groups did not reveal any significant treatment-related abnormalities. Analysis of the histopathology showed a marginal increase in the incidence of epidermal hyperplasia in females (control 2 vs treated 5). It was noted that this finding was limited to females (in males zero incidence for treated animals and controls) and was classified as no more than minimal. An increase in the incidence of endometrial gland hyperplasia in the uteri of treated females was detected, but this was considered to be a hormonal dependent event controlled by the oestrous cycle and not due to the test agent.

It should be noted that because this was meant to be a range-finding study for a potential dermal oncogenicity the degree of analysis and numbers of animals used were limited. Furthermore, the test material was applied dermally in a formulation at a concentration of 15%, which would influence the extent of exposure of the skin to the test material. However, within the limitations described previously, dermal application of a formulation containing 15% G4375 did not generated any significant signs of local or systemic toxicity in CD-1 mice treated for 5 days/week for 13 weeks. It was concluded that a concentration of 15% was suitable for a proposed oncogenicity study in this species.

Oral toxicity study: 13-26 weeks toxicity study by oral administration (gavage) in rats followed by a 4 weeks treatment-free recovery period; Centre International de Toxicologie, study no. 16539 TCR; C. Fisch; 2000; GLP/QA-yes (ref. 15).

The aim of this study was to assess the potential toxicity of G4375 (batch 95004B) following daily oral administration (gavage) to Wistar (Ico: WI [IOPS AF/Han] strain) rats for 26 weeks. Also included in this study were groups of animals kept for a 4 weeks drug-free period and additional groups that were sacrificed at 13 weeks for an interim assessment. The following table identifies the dosing protocol for the study groups.

Groups	Dose level (mg/kg/day)	Number of animals	Fate of animals and treatment duration
1. control	0	12 ♂ and 12 ♀ 8 ♂ and 8 ♀ 6 ♂ and 6 ♀ 6 ♂ and 6 ♀	26 weeks (final sacrifice) 26 weeks (for toxicokinetics) 13 weeks (interim sacrifice) 26 weeks + 4 weeks treatment-free
2. low dose	100	12 ♂ and 12 ♀ 8 ♂ and 8 ♀ 6 ♂ and 6 ♀	26 weeks (final sacrifice) 26 weeks (for toxicokinetics) 13 weeks (interim sacrifice)
3. mid dose	300	12 ♂ and 12 ♀ 8 ♂ and 8 ♀ 6 ♂ and 6 ♀	26 weeks (final sacrifice) 26 weeks (for toxicokinetics) 13 weeks (interim sacrifice)
4. high dose	1000	12 ♂ and 12 ♀ 8 ♂ and 8 ♀ 6 ♂ and 6 ♀ 6 ♂ and 6 ♀	26 weeks (final sacrifice) 26 weeks (for toxicokinetics) 13 weeks (interim sacrifice) 26 weeks + 4 weeks treatment-free

The test substance was administered by gavage as a suspension in 4% aqueous methylcellulose with 1%Tween 80. Blood sampling was performed on designated animals from all groups at 30 minutes and 24 hours after dosing in weeks 4, 13 and 26 for determination of G4375 plasma levels. Clinical signs of toxicity were monitored daily. Body weight gain was assessed on a weekly basis, while food consumption was measured weekly up to week 13 and 4 weekly thereafter. Haematology, clinical chemistry and urinalysis parameters were measured at 6, 12/13 and 26 weeks of treatment. Ophthalmological examinations were performed before the beginning of the treatment period and in weeks 12 and 25. In accordance with the schedule, designated animals were sacrificed and examination for overt macroscopic changes. Organ weight analysis was conducted. Selected tissues and tissues showing gross changes were subjected to microscopic examination. Analysis of chemical purity of G4375 was conducted at 13 weeks to check stability; stability was verified.

Plasma levels of the test agent were evident at all dosages administered over the course of the study. Plasma levels increased in a linear manner over the dose range, but not proportionally with dose. It was noted that in general plasma levels were higher in females than in males, as well as being higher in weeks 13 and 26 than in week 4. Plasma levels measured in weeks 13 and 26 were greater than those of week 4 for mid-dose females and all animals receiving the high dose; this suggests a dose- and sex- dependent accumulation of G4375. The following table presents a summary of the plasma concentration data for this study.

Week	Dose (mg/kg/day)	Sex	Concentration (ng/mL)	
			30 minutes after dosing	24 hours after dosing
4	100	Male	333	433
		Female	292	435
	300	Male	550	508
		Female	634	847
	1000	Male	689	757
		Female	984	1200
13	100	Male	309	384
		Female	472	478
	300	Male	489	542
		Female	896	1010
	1000	Male	824	733
		Female	1940	1760
26	100	Male	293	405
		Female	714	954
	300	Male	929	1250
		Female	1240	1710
	1000	Male	842	1730
		Female	2940	2780

The data presented was for maximum plasma concentration at a dose and time for each sex. Exposure, as measured by AUC, was not determined in this study. The mean plasma levels were generally slightly higher 24 hours after dosing than 30 minutes after treatment at all dose-levels, indicating a slow and sustained absorption (or reabsorption). Measurement of the controls revealed that G4375 was below the level of detection as would be expected and confirms no accidental treatment controls.

There were no treatment-related deaths or clinical signs of toxicity during the course of the study. Deaths occurring that were unrelated to treatment included 2 controls (2 females), none in the low and mid dose groups, and 2 animals (1 male, 1 female) in the high dose group. At necropsy, no major findings were observed while microscopic examination of tissues also failed to identify any specific cause of death. Since the incidence of mortality was low and at similar frequency in treated animals and controls, the cause was considered to be unrelated to treatment. It was noted that there were no overt clinical signs of toxicity that could be attributed to treatment with G4375. Observed changes included areas of hair loss, cutaneous lesions and scabs, and occasional evidence of poor condition.

Body weight gain and food consumption (includes efficiency of food utilisation) were similar across all groups, indicating no adverse effect of treatment. The indices of efficiency of food utilisation were in the range of 7.0-7.1 for all groups over the period of weeks 1-13; efficiency of food utilisation not measured after week 13. The following table shows the body weight gain (in grams) for each dose and sex over 26 weeks.

Dose (mg/kg/day)	0	100	300	1000

Sex	M	F	M	F	M	F	M	F
Weeks 1-26	362	159	371	155	360	150	363	162

There were no abnormal findings at the post-treatment ophthalmological examination. Variation in corneal thickness was seen with similar incidence in treated groups and controls. Dry keratitis (associated with exophthalmia) and corneal opacity was seen only in control animals, while a male from the high dose group had opacification of the lens in week 25.

Haematological and urinalysis parameters were unaffected by treatment. Haematological data collected at 6, 12/13 and 26 weeks were unremarkable. Observed changes in erythrocyte count, mean cell haemoglobin, activated partial thromboplastin time and lymphocyte count were slight, occurred predominantly in one sex, were not dose-related, and all the individual values were within laboratory historical background data. Results from urinalysis showed sporadic significant differences in volume of urine and specific gravity for some treatment groups when compared with controls. However, these changes were slight (marginally significant) and never dose-related.

Changes detected in clinical chemistry parameters included observations that plasma glucose levels were lower in mid-dose females (19%) and high-dose (up to 25%) males and females. Changes in glucose levels only reached significance at the week 26 analysis, but plasma glucose levels were consistently lower across the duration of the study in treated groups when compared to controls. Fluctuations in glucose of the amount described above may not be considered biological significant since the normal range varies over a range of 60-100 mg/100 mL (ref. Interpretation of diagnostic tests; J. Wallach, 3rd ed.). Reductions in glucose levels can be linked to pancreatic disorders and hepatic disease, amongst other problems. General clinical chemistry data and both macro- and microscopic examination of tissues/organs did not reveal any abnormality with either the pancreas (not assessed in organ weight analysis) or liver.

Sodium and chloride levels were slightly (2-4%), but significantly elevated (not dose-related) in both sexes at week 6, however, this effect was not seen at 12/13 or 26 weeks. It was indicated that these changes were considered not to be of toxicological significance. Inorganic phosphate was slightly (up to 15%) elevated in males at week 26 at the mid- and high doses, but not at earlier analysis times at any dose; the observed change was not dose-related. Inorganic phosphate was elevated (up to 34%) in females at week 13 at the mid- and high doses, and at the mid-dose at week 26, but none of these changes were dose-related. Fluctuations in inorganic phosphate of the amount described above may not be considered biological significant since the normal range varies over a range of 3.0-4.5 mg/100 mL (ref. Interpretation of diagnostic tests; J. Wallach, 3rd ed.).

Organ weight analysis was unremarkable and there were no treatment-related changes in organs or tissues detectable following macroscopic and microscopic examination. Apparent random changes in various organ weights were not dose-related and /or lacked similar trends in both sexes and in times of scheduled sacrifice. Macroscopic and microscopic examination revealed findings identified as commonly seen in the untreated laboratory rat of this strain and age. At 26 weeks, a greyish mass in the subcutaneous tissue of a high-dose female was identified as a mammary duct carcinoma. This finding was suggested to be consistent with spontaneous

occurring tumours in this strain of rat (Attia et al 1994). There were no findings (at 26 weeks) that could be considered related to treatment with the test material.

Endocrine disruptor (oestrogenic activity) activity of sunscreen actives was the focus of a recent study from Europe. In this study, there was no direct measurement of hormone levels to determine whether a change was induced by G4375. However, organ weight (rat uterotrophic assay) analysis did not show any increase in the size of the rat uterus in this study. It was noticed that a trend toward smaller uterus and ovary weights was evident. Furthermore, gross and microscopic examination of the uterus and ovaries of study animals revealed similar spontaneous findings across all groups. Also, liver enzyme activity, weights and structural integrity were unaffected by treatment with G4375.

Overall, it was apparent that the test material was well tolerated when given orally (by gavage) to rats over a dose range of 100, 300 or 1000 mg/kg/day for a period of up to 26 weeks. The results presented in this study were generally for group sizes of 6 to 12 animals/sex when assessing effects of the test material. These numbers are low for long-term studies, which can lead to limited study power due to increased variability (standard deviation) for the study groups.

REPRODUCTIVE TOXICITY

Study for androgenic activity by oral administration to immature, castrated rats rodent Hershberger assay; Centre International de Toxicologie [CIT]; study no. 22175 FSR; W. Gaoua; 2001; GLP/QA-yes (ref. 45).

This study examined the effect of G4375 on the weight of sex accessory glands and tissues following daily oral administration to sexually immature castrated male Sprague Dawley rats for a period of 10 days; this assay conformed to the guidelines of the OECD rodent Hershberger assay. Groups (6 animals/dose) of sexually immature castrated male rats were used in the study. The dosing protocol included 2 vehicle control groups, which were 4% methylcellulose/1% Tween 80 (vehicle for oral dosing with G4375) and corn oil (vehicle for SC injection of reference substance testosterone propionate). A total of 3 test groups were included in the study where G4375 was administered (in a volume of 10 mL/kg) by gavage at 100, 300 or 1000 mg/kg/day for 10 consecutive days. Testosterone propionate (0.4 mg/kg/day in corn oil) was used as a reference substance and was delivered in a volume of 0.5 mL/kg/day subcutaneously. General condition was assessed on a daily basis while, body weight and food consumption were recorded every 2-3 days. At completion of dosing, the animals were sacrificed and presented for necropsy. Accessory sex tissue such as the ventral prostate, seminal vesicles together with coagulating gland, levator ani and bulbocavernous muscles, glans penis and Cowpers gland were weighed. Organ weights were expressed relative to the animals body weight.

There were no unscheduled deaths during the course of study and all animals were free of clinical signs of toxicity. Body weight gain and food consumption was unremarkable, with no apparent fluctuations between groups over the course of the study. Organ weight analysis was presented in a tabulated format, which showed that testosterone propionate induced significant increases in tissues examined including the seminal vesicles (1100%↑), penis (200%↑), ventral prostate (900%↑), levator ani (300%↑) and bulbourethral glands (600%↑). The test material

G4375 did not cause a significant increase in any organ weight, with changes ranging from –6% to +29%. There were no discernible dose-relationships for changes in organ weights over the range of doses administered.

At necropsy, there were apparent differences (visual inspection) in sizes of sexual organs between the test animals and controls. Animals treated with testosterone propionate did display larger sexual organs when compared to the animals receiving G4375 and controls. It was concluded that G4375, when administered to sexual immature castrated rats at doses of 100, 300 and 1000 mg/kg/day for 10 days, was well tolerated (no signs of toxicity) and did not possess androgenic activity.

Dose range-finding study for effects on embryo-foetal development after oral administration (gavage) in the Chinchilla rabbit; RCC Ltd; study no. 753142; H. Becker; 2000 (ref.41).

Initial determination of a suitable dose range for a study does not required assessment in accordance with GLP principles. In this study, mated female Chinchilla rabbits (5/dose level) were given G4375 daily from day 6 post-coitum to day18 post-coitum at dose levels of 0, 400, 600 or 800 mg/kg/day. The vehicle consisted of 4% methylcellulose/1% Tween 80, while all substances were delivered in a volume of 4 mL/kg. Females were sacrificed on day 28 post-coitum and the foetuses removed for examination.

G4375 was well tolerated by the dams, with no evidence of clinical signs of toxicity, no deaths, no fluctuations in body weights or food consumption and no overt evidence of macroscopic changes. Reproductive parameters were consistently similar across all groups, with no evidence of an adverse effect of treatment with G4375.

Assessment of the foetuses was unremarkable, with no evidence of any treatment-related abnormalities upon external examination. Sex ratios and body weight of the offspring were similar across all groups. Based on the results from this study it was determined that a dose of up to 1000 mg/kg/day should be well tolerated by mated Chinchilla rabbits in the main study.

Dose-range finding embryotoxicity study including teratogenicity with G4375 in the rat; Research & Consulting Company; study no 607173/L'OREAL; H. Becker & K. Biedermann; 1996 (ref. 18).

The purpose of this study was to provide an initial assessment of the effects of G4375 on embryonic and foetal development in rats and establish the dose-range (for use in main study) over which these effects could occur. Study groups each containing 6 mated female Sprague Dawley rats had G4375 administered by gavage from day 6 through to day 15 post-coitum at dose levels of 0, 100, 300 or 1000 mg/kg/day. A standard dose volume of 10 mL/kg was administered and controls received the vehicle (4% methylcellulose/ 1% Tween 80). The dams were sacrificed on day 21 of gestation and the uterine contents removed and examined.

Maternal toxicity was not evident in treated animals with no deaths, no clinical signs of toxicity, no observable changes at autopsy, and no effect on food consumption or body weight gain over

the course of the study. There were no adverse effects on reproductive parameters, with treatment groups displaying similar values to the controls.

Examination of the foetuses taken at sacrifice revealed no adverse effects that could be associated with the test material. External examination, sex ratio and body weights were similar for the treated animals and controls. Total foetuses examined were 95, 103, 70 and 100 for groups receiving 0, 100, 300 or 1000 mg/kg/day; the lower value at 300 mg/kg/day was probably due to 1 female not falling pregnant. There were no foetuses found death at sacrifice in any groups and there was no evidence of foetuses being found with discernible malformation on gross examination. It was decided that dosages of 100, 300 and 1000 mg/kg/day G4375 were suitable for the main study.

Embryotoxicity study including teratology with G4375 in the rat; Research & Consulting Company; study no 607184/L'OREAL; H. Becker & K. Biedermann; 1996; GLP/QA-yes (ref. 19).

The primary focus of this study was to examine the potential effects of G4375 on embryonic and foetal development in pregnant Sprague Dawley rats. Groups of mated (paired with sexually active males) female rats (25/group) were administered G4375 by gavage once daily from day 6 through to day 15 post coitum at dose levels of 0, 100, 300 or 1000 mg/kg/day (doses based on previous dose-range finding study). The vehicle control group received 4% methylcellulose and 1% Tween 80, which was administered via the same and in the same volume as the test material. The standard dose volume was 10 mL/kg with daily adjustments for changes in weight. Dams were sacrificed on day 21 post-coitum and the uterine contents (foetuses) removed and examination according to internationally recognised criteria. Parameters monitored included mortality, clinical signs of toxicity, food consumption, body weight gain and post-mortem examination in dams. Reproductive and foetal parameters followed included implantation site, embryonic resorption, foetal resorption, dead foetuses, live foetuses, runt frequency, malformation and/or anomaly and skeletal variations.

Maternal toxicity was not evident in treated animals with no deaths, no clinical signs of toxicity and no observable changes at autopsy. Slightly (8%, noted as significant) reduced food consumption was observed in the group receiving 1000 mg/kg/day during days 6-11 of the dosing period, but this was not linked to a change in body weight gain. This finding was considered to be unrelated to treatment.

A total of 2 female rats in the 100 mg/kg/day group were found not to be pregnant. The number a rats used in the evaluation from the 1000 mg/kg/day and control groups was 24 due 1 animals in each group becoming injured/sick (unrelated to treatment with G4375). The data (all mean values) showed that the number of corpora lutea ranged from 16.9-17.9 (control 17.4), the pre-implantation loss ranged from 0.5-0.8 (control 0.8), implantation sites ranged from 16.1-17.4 (control 16.6), the post-implantation loss ranged from 0.7-1.4 (control 1.0), embryonic/foetal deaths ranged from 15-34 (control 24), embryonic resorptions ranged from 0.5-1.3 (control 1.0) and foetal resorptions occurred in the 100 and 300 mg/kg/day groups at less than 0.1% incidence. Sex ratios (male/female 53.3-54.1) and weights (on litter [5.2-5.4] and individual [5.2-5.4] basis) of live foetuses were similar in the treatment groups and controls.

Numbers of foetuses examined for abnormalities in the groups receiving 0, 100, 300 or 1000 mg/kg/day were 374, 366, 369 and 390, respectively. Mean litter sizes were 15.6, 15.9, 14.8 and 16.3 for the 0, 100, 300 and 1000 mg/kg/day groups, respectively. Detectable external abnormalities occurred in 2 (0.5%) foetuses from the 300 mg/kg/day group and 1 (0.3%) foetus from the 1000 mg/kg/day group. The types of abnormalities were rudimentary tail, caudally flexed left fore paw and caudally mal-positioned left hindleg. Skeletal examination did not reveal adverse structural changes in the foetuses that could be attributed to treatment with G4375. Observed changes such as incomplete ossification and supernumerary ribs occurred at a similar frequency in all groups.

Overall, there were no adverse effects on reproductive parameters, with treatment groups displaying similar values to the controls, which were within historical control range for this strain of rat.

Assessment of effects of treatment on the foetuses included external, visceral and skeletal examinations, and determination of sex ratios and body weights. There was no evidence of any significant adverse effects on foetal development following treatment with G4375 at doses up to 1000 mg/kg/day.

G4375, study for effects on embryo-foetal development after oral administration (gavage) in the Chinchilla rabbit; Research & Consulting Company; study no 753153; H. Becker & K. Biedermann; 2001; GLP/QA-yes (ref. 42).

The primary focus of this study was to examine the potential effects of G4375 on embryonic and foetal development (from implantation to closure of the hard palate) in pregnant Chinchilla rabbits. Groups of mated (paired with sexually active males) female rats (20/group) were administered G4375 by gavage once daily from day 6 through to day 18 post coitum at dose levels of 0, 100, 300 or 1000 mg/kg/day (doses based on previous dose-range finding study). There were additional groups of 5 animals/dose to be used for toxicokinetic evaluation. The doses used in this study were based on a dose-range finding study where rabbits showed no adverse effects at doses up to 800 mg/kg/day. As a result of total litter loss by resorption in a number of animals across all groups surviving litters numbered 14, 12, 12 and 14 for the 0, 100, 300 and 1000 mg/kg/day dose groups, respectively. These were below the recommendations of the ICH guidelines, which aimed at 16 to 20 litters/group. Therefore, it was decided to include litters from the toxicokinetic groups to raise the litter numbers for the main study.

The vehicle control group received 4% methylcellulose and 1% Tween 80, which was administered via the same and in the same volume as the test material. The standard dose volume was 4 mL/kg with daily adjustments for changes in weight. Dams were sacrificed on day 28 post-coitum and the uterine contents (foetuses) removed and examination according to internationally recognised criteria.

Parameters monitored included mortality, clinical signs of toxicity, food consumption, body weight gain and post-mortem examination in dams. Reproductive and foetal parameters followed

included implantation site, embryonic resorption, foetal resorption, dead foetuses, live foetuses, runt frequency, malformation and/or anomaly and skeletal variations.

It was noted that there were no observable adverse effects in the dams following treatment with G4375 at doses up to and including 1000 mg/kg/day. Food consumption, body weight gain, clinical signs of toxicity and findings at necropsy were similar across all treatment groups and controls. There were 2 deaths (1 sacrificed in extremis; 1 found dead with incomplete collapsed lungs, which were discoloured reddish) in the group receiving 300 mg/kg/day and 1 death at 1000 mg/kg/day (due to intubation error). Food consumption was generally reduced in treatment groups compared with controls, but this was consistent with pre-dosing findings/trends. The body weight gain was similar across all groups. At necropsy, a common finding of hairs or crateriform areas in the stomachs of some females in all groups gave no indication of being related to treatment with G4375.

Reproductive parameters were unaffected by treatment with G4375. The number of females with live foetuses at termination (includes main and toxicokinetic animals) were 18, 16, 16 and 18 at 0, 100, 300 and 1000 mg/kg/day, respectively. It was noted that in the females with live foetuses at termination had a similar degree of post-implantation loss (mean range 0.8-1.1, control 0.9) and number of live foetuses (range 125-168, control 151). Dams displayed similar numbers of corpora lutea (mean range 10.2-10.9, control 10.8), pre-implantation loss (mean range 7.1-13.9, control 13.9), implantation sites (mean range 86.1-92.9, control 86.1), embryonic resorptions (mean range 0.2-0.6, control 0.6) and foetal resorptions (mean range 0.3-0.6, control 0.3). There were no dose-related trends for changes in any of the parameters.

It was noted that there were no clear (significant) findings indicating an effect of treatment on the development of the test animals. However, there was an increased incidence of missing kidney and ureter observed in foetuses from the 300 and 1000 mg/kg/day groups when compared with controls. Sex ratios (female/male 50.3-64.0, control 51.7) did fluctuate in the 300 mg/kg/day group from the other groups, but there was no dose-related trend and this effect was seen as incidental since it was within the normal range for this strain of rabbit. Body weights (on litter [31.9-33.6] and individual [31.5-32.1] basis) of live foetuses were similar in the treatment groups and controls.

Data on the frequency of foetuses with external and visceral abnormalities showed the following. It was noted that most of the abnormalities concerned foetuses with low body weight and they were considered not related to treatment. Incidences of flexure of one or both of the forepaws were 1, 1, 0 and 2 for the 0, 100, 300 and 1000 mg/kg/day groups, respectively. Domed head was observed in 3 foetuses from the 1000 mg/kg/day group, along with abnormal facial appearance in a further foetus. Acephalia (absence of head) was observed in 1 foetus, but this change was identified as an occasional spontaneous malformation in this strain of rabbit. Missing kidney and ureter were noted in 0, 0, 2 foetuses (1 litter) and 4 foetuses (3 litters) in the 0, 100, 300 and 1000 mg/kg/day groups, respectively.

An assessment of these findings identified a historical control value of less than 1% (historical value 7/931, 0.75%) for an absence of kidney and ureter in foetuses in this strain of rabbit. Considering that the 4 foetuses (2% of total examined) were from 3 separate litters and this effect

did not correlate with a decreased foetal body weight, the possibility that this effect was related to treatment could not dismissed.

Skeletal development was assessed with unremarkable findings. It was noted that the types of abnormalities and their frequency of incidence were consistent with the normal range seen in historical control data for this strain of rabbit. Findings included thoracic vertebral bodies fused, ribs fused, sternebrae fused/bipartite or abnormally shaped, and thoracic vertebral body, arch and rib missing. In treatment groups, there was an increased incidence of incompletely or unossified bones of the fore- and hind- paws, however, the incidences were not dose-dependent and were within historical control values.

Overall, there were no adverse effects on maternal condition at doses of up to 1000 mg/kg/day G4375 delivered by gavage during organogenesis (days 6 to 18 of gestation) in the Chinchilla rabbit. In general, the occurrence of either external, visceral or skeletal abnormalities were similar across all groups, with no evidence of dose-relationships and frequencies falling within historical control values. However, the incidence of absence kidney/s and ureter/s was just outside the historical range and occurred in uncompromised animals. This is an equivocal result for developmental changes in the Chinchilla rabbit.

G4375, study for effects on embryo-foetal development after oral administration (gavage) in the Chinchilla rabbit; Research & Consulting Company; study no 813688; H. Becker, A. Marburger & K. Biedermann; 2002; GLP/QA-yes (ref. 43).

The primary focus of this study was to examine the potential effects of G4375 on embryonic and foetal development (from implantation to closure of the hard palate) in pregnant Chinchilla rabbits. Groups of mated (paired with sexually active males) female rats (30/group) were administered G4375 by gavage once daily from day 6 through to day 18 post coitum at dose levels of 0, 300 or 1000 mg/kg/day (doses based on previous study). The number of females used per group was increased to assist in the interpretation of the result obtained in the previous study (ref. 42), where foetuses exposed to 300 and 1000 mg/kg/day G4375 had a slightly elevated incidence of missing kidney/s and ureter/s.

The vehicle control group received 4% methylcellulose and 1% Tween 80, which was administered via the same route and in the same volume as the test material. The standard dose volume was 4 mL/kg with daily adjustments for changes in weight. Dams were sacrificed on day 28 post-coitum and the uterine contents (foetuses) removed and examination according to internationally recognised criteria.

Maternal tolerability was good, with no clinical signs of toxicity and no adverse effect on body weight gain. Two dams receiving 300 mg/kg/day were found dead, one each on the morning of days 7 and 17 post-coitum. These deaths were considered incidental since there were no deaths in the higher dose group and these animals had shown no clinical signs of toxicity prior to their death; deaths likely to be due to intubation errors. Mating performance data showed that 3, 3 and 2 females were found not to be pregnant from groups receiving 0, 300 and 1000 mg/kg/day, respectively. The total number of females with live foetuses at termination was 25, 24 and 28 at 0, 300 and 1000 mg/kg/day, respectively.

Food consumption at 1000 mg/kg/day was slightly decreased (9.7% compared with controls) during the course of the treatment period and remained lower (approx. 8%) than controls until sacrifice. However, this reduction in food consumption was not accompanied by a decreased body weight gain.

Reproduction data were unremarkable for any adverse effects associated with treatment with G4375. Parameters such as pre-implantation loss, implantation sites, post-implantation loss, implantation site scars, embryonic/foetal deaths and embryonic resorptions all showed a positive/beneficial effect of treatment at 1000 mg/kg/day, and to a lesser extent at 300 mg/kg/day.

The total numbers of foetuses examined for abnormalities were 212, 224 and 285 at 0, 300 and 1000 mg/kg/day, respectively. There were no dead foetuses detected at sacrifice and only 1 foetus from the 300 mg/kg/day group presented with an abnormality. The body weights of the live foetuses were similar in the 300 mg/kg/day group and controls, while the 1000 mg/kg/day foetuses had a slightly lower litter and individual body weight (approx. 5-6%). Sex ratios were similar across all groups.

External and visceral examination revealed 1 control foetus had its right eye lens completely opaque (not considered an abnormality), while 1 foetus from the 300 mg/kg/day group had an encephalocele (congenital gap of the skull with herniation of brain substance) affecting the region of the parietal bone. Foetuses from the 1000 mg/kg/day group did not display any abnormalities on examination. There was no evidence of the previously identified finding of missing kidney/s and ureter/s seen in the same strain of rabbit over the same dose range (300-1000 mg/kg/day).

Overall, a dose of up to 1000 mg/kg/day G3275 was well tolerated by the both the pregnant rabbits and developing offspring.

G4375: study on fertility and early embryonic development to implantation by oral route (gavage) in rats; Centre International de Toxicologie; study no. 19479RSR; C. Fabreguettes; 2001; GLP/QA-yes (ref. 48).

In this fertility study, groups of 24 male and 24 female Wistar Han rats received G4375 (batch DEF/C95004B) by the oral route (gavage) at dose levels of 0, 100, 300 or 1000 mg/kg/day according to the following protocol. In males, dosing commenced 29 days prior to mating and continued through the mating period until sacrifice. In females, dosing commenced at 15 days pre-mating and continued throughout mating and up until day 7 post-coitum. The vehicle control group received 4% methylcellulose and 1% Tween 80, which was administered via the same route and in the same volume as the test material. The standard dose volume was 8 mL/kg with daily adjustments for changes in weight. Parameters examined during the study included clinical signs of toxicity, mortality, body weight and food consumption, mating and fertility data (including sperm parameters, oestrus cycle and reproductive data), macroscopic examination of parent animals and organ weights.

The condition of the parent generation during the course of this study was unremarkable, with no clinical signs of toxicity and no deaths. Clinical signs of toxicity observed included hair loss (8 individuals total, 4 from control group), nodules on the head (1 HD male) and a reddish discharge (1 HD female). Body weight gain and food consumption were similar in the 100 and 300 mg/kg/day groups and controls, while the high dose females displayed a slightly lower food consumption (5-10%) and significantly lower body weight gain (31%) during the pre-mating period.

Mating and fertility data indicated that the pre-coital time was similar in all groups (between 2.4 to 4.0 days), the mating index was 100% in all groups, the gestation index was 100% in all female groups and the fertility index was similar (83% to 93%, no dose-relationship) across all groups.

Litter data (mean values) showed that parameters such as corpora lutea (14.3-15.4), implantation sites (12.0-13.9), pre-implantation loss (10-16), resorptions (0.5-1.5), dead foetuses (0), live foetuses (11.5-12.6) and post-implantation loss (5-11) were similar (no significant differences or dose-related trends) for all groups. The only parameters that showed significant effects were number of live concepti (increased) and post-implantation loss (decreased), where the high-dose was shown to be beneficial.

Investigation into the oestrus cycle found that it was not adversely affected by treatment with G4375 at any dose used in this study. There was no effect of treatment on the assessed sperm parameters (count, viability, motility and morphology) at the dose levels used in this study.

There were no treatment-related macroscopic findings at necropsy for any of the parent generation. Evaluation of organ weights was unremarkable, with no evidence of changes in organ associated with reproduction.

Overall, G4375 was well tolerated at doses up to 1000 mg/kg/day in the rat. There was no evidence of any adverse effects on fertility of both the male and female rat.

G4375: study on effects on pre- and post-natal development by oral route (gavage) in rats; Centre International de Toxicologie; study no. 19480RSR; J. Richards; 2001; GLP/QA-yes (ref. 51).

This study examined the effect of G4375 on pregnant and lactating Wistar Han rats (F0 generation), and on their developing embryo/foetus through to sexual maturity (F1 generation). G4375, at dose levels of 0, 100, 300 or 1000 mg/kg/day, was administered orally (gavage) daily to groups of 24 mated females from implantation up to weaning (day 6 post-coitum to day 21 post-partum) of the F1 generation. The vehicle control group received 4% methylcellulose and 1% Tween 80, which was administered via the same route and in the same volume as the test material. The standard dose volume was 8 mL/kg with daily adjustments for changes in weight. The F0 generation was monitored daily for clinical signs of toxicity and mortality, while data on food consumption and body weight gain were recorded at regular intervals. The F0 generation delivered their offspring naturally and pregnancy and litter data were recorded.

Daily observations of clinical signs and mortality of litters was carried out during lactation, while body weights were recorded at regular intervals. On day 4 post-partum the size of each litter was adjusted to 8 pups (4/sex where possible). Development of these pups was followed through the measurement of a series of parameters. On day 22 post-partum a male and female from each litter were selected to form the F1 generation (19-20 animal/sex/dose group). The F1 generation was monitored for clinical signs of toxicity and mortality, while body weight and food consumption were assessed on a weekly basis; included was an assessment of sexual development of both males and females. Also studied were neuro-behavioural parameters including auditory, visual, learning and memory functions, as well as spontaneous locomotor activity. Upon reaching sexual maturity F1 generation males and females were paired (from same dose group) and the reproductive performance of these animals was assessed.

Data from the F0 generation showed there were no treatment-related clinical signs of toxicity or deaths during their study phase. During pregnancy, 1 female from each group displayed clinical signs such as diffuse alopecia and the presence of blood in the vagina. During lactation, between 1 to 3 females from each group showed clinical such as alopecia, emaciation or nodules on the tail (seen in control animal). These clinical signs are commonly observed in this strain of rat and based on the low frequency, and absence of a dose-relationship, were considered not to be treatment-related.

A reported death of 1 pregnant control female was noted as a spontaneous occurrence; the animal had thick reddish contents in both horns of the uterus. Animals not delivering by day 25 post-coitum were sacrificed and examined (either not pregnant or had litter resorption); 2 females from the 100 mg/kg/day and 3 from the 1000 mg/kg/day were in this category. During the lactation period a female from the 300 mg/kg/day group was sacrificed due to poor condition, which were characterised by emaciation, coldness to the touch, piloerection, hunched back, hypotonia, pallor of the extremities, dyspnea and pale eyes. At necropsy, this animal displayed evidence of having abnormal a spleen, liver, uterus and vagina. It was reasoned that the problems observed in this animal were not due to treatment since there was no evidence of these adverse effects at the higher dose of 1000 mg/kg/day (no dose-relationship).

A slight reduction (less than 10% at high dose during pregnancy or lactation phases) in body weight gain was noted during the gestation/lactation period in treated females. It was stated that these changes were slight (generally not significant), did not involve all animals in the groups, were not dose-related and were also observed in some control animals. It was concluded that a relationship to treatment could not be discounted (was likely), but the changes were considered not to be of toxicological significance. A similar finding was noted for food consumption, with slight reductions across the treatment groups that were not dose-related and unlikely to be of toxicological significance.

Reproductive parameters such as gestation index (91-100%), gestation length (21.5-21.8 days), delivery data such as litter size (9.2-11.1, control 10.0) and sex ratio (42.4-53.5% males), implantation sites (10.8-11.7), post-implantation loss and neonatal losses were similar in treatment groups and controls, indicating no adverse effects of G4375. The range of values seen for the sex ratio was within the laboratory control range. At birth there were no observable macroscopic abnormalities in any of the pups.

Assessment showed that the survival at birth and development of pups during the lactation period was unaffected by treatment with G4375. There were an increased number of pups from the 100 and 300 mg/kg/day groups dying in the lactation period, while the 1000 mg/kg/day group had the lowest mortality rate. Survival in the 0, 100 and 1000 mg/kg/day groups was considered to be similar with pups dying at a rate of 2, 5 and 1, respectively. Pup deaths in the 300 mg/kg/day group were elevated (15) due to the loss of 2 litters, one of which was due to the sacrifice of the dam for humane reasons.

There were no clinical signs of toxicity in pups from any of the treatment groups. Clinical signs seen across all groups included scattered hair, scabs, cutaneous lesion and cold to touch, swollen abdomen, emaciation, dyspnea; all these signs were transient in nature and presence at a low frequency. Body weights of pups from all treatment groups were reduced from day 14 post-partum. This effect was described as slight and was suggested to be linked to the observed reduced food consumption of the F0 dams. Interestingly, the body weight reduction seen across all treatment groups could be described as a reverse dose-relationship. It was noted that the low dose animal (30.4 g) were the smallest relative to the controls (40.4 g), the mid-dose group (34.6 g) was next while the high dose group (36.7 g) showed a marginal reduction in body weight (<10%).

At necropsy, there were no treatment-related abnormal macroscopic findings detected in either the F0 dams or their pups sacrificed at the end of the lactation period. Examination of the F1 generation revealed abnormalities (such as enopthalmos, accentuated lobular pattern of the liver, liver reduced in size, liver enlarged, dilated pelvis of the kidney, yellowish nodule on epididymis, small epididymis, epididymis translucent, testis translucent, testis enlarged) that are commonly found in rats of this strain. The frequency of abnormalities mentioned above was similar across all groups.

Data from the F1 generation indicated that there were no adverse effects on food consumption, body weight gain or sexual development during pre-mating and pregnancy periods for these animals. Furthermore, the F1 animals did not present with any clinical signs of toxicity during this period. General physical development was identical for all groups as assessed by number of pups (10%) in which pinna unfolding, hair growth, tooth eruption, eye opening and auditory canal opening occurred on scheduled day. Development of spontaneous reflex actions in the treated pups was similar to controls, with surface righting, cliff avoidance and air-righting reflex shown to occur (98.8-100%) on expected day. Neuro-behavioural assessment of the F1 generation by measuring parameters such as auditory, visual learning and memory functions, as well as spontaneous locomotor activity showed these were all unaffected by treatment with G4375. It was noted that the learning function of high dose females (not males) took longer (47.5 vs 58.1 seconds) than the controls, but this was only seen in females and was not dose-related. Both high dose females and males performed better than controls at the memory test.

There was no discernible adverse effect of treatment on fertility parameters [mating index (100% all groups), pre-coital time (2.9-3.6 days), fertility (80-95%) and gestation indices (100% all groups)] or parameters associated with pregnancy. Parameters such as implantation sites (12.3-13.3), pre-implantation loss (29-47, control value 47), live concepti (11.3-12.1), resorptions (0.8-

1.2) and post-implantation loss (0.8-1.3) were similar for all groups. Overall, total pre- and post-implantation loss was 23.8, 24.3, 24.7 and 22.2% for the 0, 100, 300 and 1000 mg/kg/day groups, respectively. Sexual development of males (preputial separation) and females (vaginal opening) occurred at similar times (35-36 days in males, 34-35 days in females) in treated animals and controls. Necropsy of the F1 animals was unremarkable, with no treatment-related adverse effects detected on examination.

Overall, it would appear that a dose of up to 1000 mg/kg/day G4375 had minimal effect on the reproductive cycle of Wistar Han rats when administered from implantation through lactation and weaning of F1 pups.

GENOTOXICITY

Mutagenicite du produit G4375 RF004 – L'oreal; Central Product Safety Depart. L'Oreal; study no. LLB/JK 931710/505; M. Shahin & J. Leclaire; 1994; GLP/QA-no evidence of status (ref. 20).

An English summary was presented for a mutagenicity testing G4375. The same information was presented in French. The summary consisted of a structure of G4375, a single line results statement and 3 pages of tables. The result statement indicated that in both the absence and presence of metabolic activation, G4375 yielded negative results on the strains used. The tables indicated that this was the assessment of G4375 in strains (TA1535, TA100, TA1537, TA1538 and TA98) of *S. typhimurium* in the absence and presence of metabolic activation. Tabulated data indicated that vehicle and positive (DNB & 2AA) controls were included. G4375 was tested over a concentration range of 10-5000 μg/plate and was negative over the entire concentration range. The information presented did not include cell viability testing in the presence of G4375.

Recherche de mutagenicite sur Salmonella typhimurium His- selon la technique de BN Ames sur le produit G4375 Essai de screening; Institut Pasteur de Lille; study no. IPL-R931016/G4375/L'Oreal; D. Marzin; 1993; stated conducted according to OECD and EEC guidelines (ref. 21).

A full English version was presented for a mutagenicity test of G4375 using the Ames protocol. The same information was presented in French. This study examined the potential mutagenic activity of G4375 in strains (TA100, TA1537 and TA98) of *S. typhimurium* in the absence and presence of metabolic activation in two independent tests. The concentration range used in the dose-range finding part of the assay was 1.5-5000 μg/mL. Data generated from preliminary testing resulted in a range of 0.5-1500 μg/plate being selected for use in the main assay; this range was based on preliminary cytotoxicity testing and determination of precipitation of test material at concentrations used in the assay. Assay protocol included metabolic activation, which was achieved using a rat liver S9 fraction. Positive (9-aminoacridine, 2-anthramine, 2-nitrofluorene & sodium azide) and vehicle (DMSO) control groups were included in the assay.

In this assay, testing of G4375 in the presence and absence of metabolic activation did not result in a change in revertant frequency of biological significance for any of the strains tested. There was a slight but significant increase in revertant frequency in the TA100 strain, however, this

was not dose-related and did not satisfy the criteria (less than doubling of revertant frequency, approx. 50% increase) for a positive effect. Furthermore, it only occurred in one of the two independent assays. G4375 was not mutagenic in this assay.

Salmonella typhimurium and Escherichia coli reverse mutation assay with G4375; RCC; Study No. 607410; H. Wollny; 1996; GLP/QA-yes (ref. 22).

This study assessed the potential for G4375 (batch 95003/B) to induce gene mutations in S. typhimurium strains (TA1535, TA1537, TA98 & TA100) and E. coli strains (WP2 & WP2uvrA) in the presence and absence of metabolic activation (rat liver fraction). Concentrations of 33.3, 100.0, 333.3, 1000.0, 2500.0 and 5000.0 μg/plate G4375 were tested in duplicate independent assays. Positive (NaN_3, 4-NOPD, MMS & 2-AA) and vehicle (DMSO) controls were included in the study. Cytotoxicity was not evident over the range of concentrations used in this study.

There were no significant increases in revertant colony numbers over the range of concentrations of G4375 tested. Appropriate results were obtained for the positive controls, which validated the assay. It was concluded that G4375 was considered non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay in presence and absence of metabolic activation.

Gene mutation assay in Chinese Hamster Ovary (CHO) cells in vitro with G4375; RCC; Study No. 607094; H. Wollny; 1996; GLP/QA-yes (ref. 23).

This study assessed the potential for G4375 (batch 95003/B) to induce gene mutations at the HRPT locus in Chinese hamster ovary cells in the presence and absence of metabolic activation (rat liver fraction). Duplicate independent assays were conducted using the test material at concentrations of 1, 3, 10, 30, 50 or 80 μg/mL in the presence and absence of metabolic activation. The highest concentration used in the assays was on the limit for the appearance of significant precipitation, while moderate (up to 23%) cytotoxicity occurred in the absence of metabolic activation (none in presence of metabolic activation). Positive (ethylmethanesulfonate & 7,12-dimethylbenz(a)anthracene), negative and vehicle (acetone) controls were included in the study.

In the duplicate assays, there was no evidence of mutagenic activity over the concentration range (1-80 μg/mL) of G4375 in either the presence or absence of metabolic activation. Results generated (significant increase in mutant frequency) from the positive controls provided validation for the assay. It was concluded that G4375 did not induce gene mutations at the HPRT locus in CHO cells.

Chromosome aberration assay in Chinese Hamster V79 cells in vitro with G4375; RCC; Study No. 607421; A. Czich; 1996; GLP/QA-yes (ref. 24).

This study assessed the potential for G4375 (batch 95003/B) to structural chromosomal aberrations in V79 cells of the Chinese hamster in two independent assays in the presence and absence of metabolic activation. Positive (EMS & CPA), negative and vehicle (acetone) controls were included in the study. Preliminary testing for toxicity and precipitation in solution was

carried out. Precipitation was seen at concentrations higher than 50 μg/mL. Cytotoxicity was examined at concentrations above solubility limits (up to 5000 μg/mL). At these limits, there was no evidence of cytotoxicity in the absence of metabolic activation, while there was a slight dose-related cytotoxicity in the presence of metabolic activation. Dose levels used in the study were from 3-5000 μg/mL in the presence and absence of metabolic activation. In both assays, cytogenetic damage was assessed following cell fixation intervals of 18 and 28 hours and in relation to degree of precipitation.

Results from both assays and fixation times were negative, indicating no biologically relevant increases in cells with structural aberrations following exposure to G4375 at concentrations up to 5000 μg/mL in the presence or absence of metabolic activation. The positive controls caused significant increases in the incidence of structural chromosomal aberrations (gaps or exchanges), thus validating the assay. The mitotic index (as a percentage of control) was determined and was found to fluctuate within expected variability without displaying any dose-related trends.

Micronucleus assay in bone marrow cells of the mouse in vivo with G4375; RCC; Study No. 607105; W. Volkner; 1996; GLP/QA-yes (ref. 25).

This assay examined the potential for G4375 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. Animals numbers in this study 10 (5/sex) per test group (time and dose). The test agent (G4375) was administered orally following preparation in an aqueous vehicle containing methocel (1%) and Tween 80 (1%). Delivery volume was 10 mL/kg. Dose levels used were 200, 670 or 2000 mg/kg for a 24 hour exposure period and 2000 mg/kg for a 48 hour exposure period; dosing frequency was a single administration. At 24 and 48 hours after treatment, bone marrow cells were collected for micronucleus analysis. PCE numbers used in the analysis were 1000 to 2000 per animal. Cytotoxicity assessment was based on the ratio of PCE to normochromatic erythrocytes (NCE), which was expressed as the number of NCE/1000 PCE.

It was noted that the highest dose used did not elicit any adverse effects in the animals. The ratio of PCE to NCE was consistent for the treated groups and vehicle controls indicating no treatment-related cytotoxicity toward bone marrow cells. Analysis of the frequency of micronuclei revealed percentage PCE's with micronuclei ranged from 0.02-0.07 (not dose-related), compared to the vehicle control with a value of 0.03 over the 24 hour exposure period. Cyclophosphamide (positive control) generated a significant increase in micronuclei with a value of 0.68 over the same exposure period. It was concluded that G4375 did not induce an increase in the frequency in micronuclei in mouse bone marrow cells; it was considered not to be mutagenic in this assay.

Chromosome aberration assay in vitro. Photo-mutagenicity in Chinese Hamster Ovary (CHO) cells with G4375; RCC; Study No. 607138; A. Czich; 1996; GLP/QA-yes (ref. 27).

G4375 was assessed for its ability to induce structural chromosomal aberrations in cultured CHO cells following UV irradiation. The test material was prepared in acetone prior to its use in two independent experiments. Preliminary testing was used to determine the appropriate concentration of G4375; this was based on solubility and cytotoxicity. A concentration range of

1.0-10.0 μg/mL (concentration in CHO culture) was used in the main assays, which was based on solubility limits and not cytotoxicity (none observed). A positive control (8-methoxypsoralen) was included in the experiments to validate the effectiveness of the procedure.

There were two irradiation protocols used, with 200 mJ/cm^2 UVA and 12 mJ/cm^2 UVB used in experiment 1 and 300 mJ/cm^2 UVA and 18 mJ/cm^2 UVB used in experiment 2. In both experiments irradiation was given 30 minutes after application of the test material. Exposure levels of irradiation were based on CHO cell sensitivity to UV light, which was determined in a preliminary assay. The lower level of irradiation was at the threshold of an increase in induced structural chromosomal aberrations, while the higher level of irradiation did induce a slight but distinct increase in the frequency of structural chromosomal aberrations. Preparation of the chromosomes for analysis was carried out 22 hours and 30 hours after commencement of treatment with G4375.

In both experiments there was no evidence that G4375, over the dose range of 1.0-10 μg/mL, induced a reduction in the mitotic indices of cultured CHO cells. Analysis of aberrant CHO cells at both 22 and 30 hours with no irradiation showed a range (type and frequency) of chromosomal aberrations that were similar in test and vehicle control cell cultures. There was one instance where a statistically significant increase in a particular type of aberration (excl gap) was observed, but it occurred at the mid-concentration (not dose-related) of G4375 and in the 22 hours analysis group only. This result was of no biological significance.

Analysis of aberrant CHO cells at both 22 and 30 hours with low level irradiation showed a range (type and frequency) of chromosomal aberrations that were similar in test and vehicle control cell cultures. There was one instance where a statistically significant increase in a particular type of aberration (excl gap) was observed, but it occurred at the low-concentration (not dose-related) of G4375 and in the 22 hours analysis group only. This result was of no biological significance.

Analysis of aberrant CHO cells at both 22 and 30 hours with high level irradiation showed a range (type and frequency) of chromosomal aberrations that were similar in test and vehicle control cell cultures. There was no evidence of elevated aberration count, with test values lower than corresponding vehicle control values.

Concurrent positive control groups showed a distinct increase in cells displaying structural chromosomal aberrations, thus enhancing/establishing the validity of the test procedure.

Photomutagenicity in Escherichia coli. Reverse mutation assay with G4375; RCC; Study No. 607116; H. Wollny; 1996; GLP/QA-yes (ref. 26).

This assay examined the potential for G4375 (batch DEF/C95003/B) to induce reverse gene mutations in the presence and absence of UV radiation (similar to sunlight) using the Escherichia coli strain WP2. G4375 was tested at concentrations of 33.3, 100.0, 333.3, 1000.0, 2500.0 and 5000.0 μg/plate. Preliminary testing showed that cytotoxicity was not evident over this dose range. Three independent experiments (performed in triplicate) were carried out involving plate incorporation and pre-incubation testing. Optimal UV radiation intensity conditions for E. coli

were established during pre-testing as 9 mJ/cm^2 UVA and 1 mJ/cm^2 UVB. This dose of UV radiation was identified as not strongly increasing the background mutant frequency of E. coli. A positive control substance (8-methoxypsoralen at 125 μg/plate) was included in the assays.

The test procedure involved the addition of G4375 to cultures/plates of E. coli over the stated concentration range prior to UV irradiation of the plates for 10 seconds. A similar approach was taken for positive, negative and vehicle control groups.

There was no evidence of consistent, dose-related increases in the frequency of revertant colonies over the concentration range of G4375 following exposure to UV radiation identified as artificial sunlight. The negative control group had revertant (revertant/plates) values (139-263) consistent higher than those seen in the test groups (87-200), while the positive control had values distinctly greater (308-420) than either the negative or test groups. The vehicle groups had revertant values in the same range as those seen for the test groups.

It was concluded that G4375 was not mutagenic in this photomutagenicity assay, where cultures of E. coli were exposed to UV radiation (described as artificial sunlight) and G4375 over a concentration range of 33.3-5000.0 μg/plate.

CLINICAL DATA/INFORMATION

Human repeat insult patch test for contact sensitisation; Quintiles Consumer Product Evaluation; Study No. LRL/117/V; C. Ponti; 1996; GCP/QA-yes (ref. 35).

It was noted that the methodology use in this study was developed by Marzulli and Mailbach (Phototoxicity [photoirritation] of topical and systemic agents; Dermato-toxicology; 3rd edition; des. Marzulli-Mailbach; Hemisphere Public Corp. 431-440; 1987).

This study examined the skin irritancy and skin sensitisation potential of G4375 (lot 95002A) in 50 human volunteers; this study was a multiple assessment process with 9 other agents tested at the same time. G4375 was tested as a 15% w/v solution in acetone. Filter paper discs were dipped into the test solution before being placed in Finn chambers that were held on surgical tape. Exposure to the test material was achieved by attaching the Finn chambers to the skin of the subjects. It was noted that 25 of the subjects were identified as atopic, which suggests that half the subjects were naturally sensitised towards allergic reactions. The study involved three 47 hour induction exposures, which was followed by a 2 weeks rest period. After the rest period duplicate 47 hour challenge exposures were conducted at both the original and naïve sites. The skin was assessed for reactions at 48 and 72 hours after each induction (for skin irritation) and at 48 and 96 hours after challenge (for skin sensitisation).

A total of 47 subjects completed the study. One of these subjects was withdrawn following the prescription of anti-inflammatories by her GP (unrelated condition) and two were unable to attend for personal reasons. Overall, there were no serious adverse events/effects resulting from treatment.

A table of induction assessment points/numbers (total 9) was presented displaying the individual reaction data for skin irritation and skin sensitisation. A total of 35/47 subjects showed no evidence (no visible relevant reaction) of skin irritation during induction, while the remaining 12 had slight but distinct erythema at 1 or 2 of the 9 assessment points only. These reactions were described as minimal and infrequent in occurrence indicating G4375 (in acetone) could be described as a marginal skin irritant. It is possible that the use of acetone as the vehicle could have enhanced the skin reactivity of G4375.

At challenge, a total of 5/47 subjects displayed slight erythema at a similar intensity to that seen during the induction phase. None of the remaining 42 subjects showed any sign of skin reaction. It was noted that 3/5 of the subjects showing slight erythema at challenge had a reaction during the induction process. This data indicates that G4375 was not a skin sensitiser in normal and atopic subjects tested in a repeat insult patch test.

Assessment of the phototoxicity potential of a sun protection formulation by the method of Kaidbey and Kligman; Centre Pharmacologie Clinique Appliquee Dermatologie; Study No. CPCAD CPC 3033; J. Ortonne & L. Duteil; 1998; GCP-yes (ref. 36).

It was noted that the methodology used in this study was developed by Kaidbey and Kligman (Identification of topical photosensitising agents in humans; J. Invest. Dermatol.; 70; 149-151; 1978).

This study examined the phototoxic potential of a sunscreen formulation (containing 15% G4375) in 12 healthy subjects (8 females & 4 males) aged between 18 and 49 years with type III skin. Each of the 12 subjects was pre-tested to determine their minimal erythema dose (MED) following exposure to UV radiation. Phototoxicity assessment was carried out using both the sunscreen formulation with active and the vehicle formulation alone. The test substances were applied (50 $\mu L/cm^2$) on either side of the lumbar area of the back, covered with an occlusive dressing, which was left in contact with the skin for 24 hours. A control (empty) patch was applied to roughly the same area as the test patches. Following removal of the patches, sites of application were irradiated with 20 J/cm2 of UVA in conjunction with a 0.75 MED (UVA + UVB) dose of radiation; UV radiation generated by an artificial light source (1000 W xenon arc solar stimulator with appropriate filters). The radiation output was measured by a radiometer (3D-600). All sites used in the study were examined at 1, 24, 48 and 72 hours after irradiation.

The results showed that the sunscreen containing G4375 and its vehicle formulation did not cause any phototoxic reactions under the conditions described above. Post-irradiation assessment of the test sites at 1, 24, 48 and 72 hours found no skin reactions of a phototoxic nature in any of the 12 subjects.

Assessment of the photosensitisation potential of a sun protection formulation by the method of Kaidbey and Kligman; Centre Pharmacologie Clinique Appliquee Dermatologie; Study No. CPCAD CPC 3034; J. Ortonne & L. Duteil; 1998; GCP-yes (ref. 37).

It was noted that the methodology used in this study was developed by Kaidbey and Kligman (Identification of topical photosensitising agents in humans; J. Invest. Dermatol.; 70; 149-151; 1978).

This study examined the photosensitising potential of a sunscreen formulation (containing 15% G4375) in 30 healthy subjects (20 females & 10 males) aged between 19 and 51 years with type II or III skin. Each of the 30 subjects was pre-tested to determine their minimal erythema dose (MED) following exposure to UV radiation. Photosensitisation assessment was carried out using both the sunscreen formulation with active and the vehicle formulation alone. During a 3 weeks induction phase, 50 $\mu L/cm^2$ of the test articles were applied twice a week to an area of the lumbar region of the back, covered with an occlusive dressing and left in contact with the skin for 24 hours. An untreated occluded site acted as a control area. Each time the patches were removed (after 24 hours), the 3 test sites were irradiated with a dose of UV (A&B) equivalent to 3 times the MED.

Challenge proceeded after a 2 weeks rest period. At challenge, each test article was reapplied under occlusion for 24 hours on 2 test sites located on either side of the back. Two untreated but occluded sites were included in protocol. Following removal of the challenge patches, the test sites were irradiated with 5 J/cm2 of UVA in conjunction with a 0.75 MED (UVA + UVB) dose of radiation; UV radiation generated by an artificial light source (1000 W xenon arc solar stimulator with appropriate filters). The radiation output was measured by a radiometer (3D-600). All sites used in the study were examined and scored at 24, 48 and 72 hours after the challenge irradiation.

At challenge, the post-irradiation examinations of the test sites at 24, 48 and 72 hours after irradiation did not reveal any skin reactions of a photoallergic type in any of study subjects; all subjects completed the study. Tabulated data identifying adverse events for the subjects undertaking the study revealed minor problems, which were considered not to be related to the test substances. A total of 6 subjects were reported to have experienced the symptoms of a cold (2), influenza (1) or a headache (3) during the course of the study. All these conditions were resolved and none of the subjects were forced to quit the study. Data presented for the induction period showed a protective effect of the sunscreen formulation, as evidenced by lower scores compared to sites treated with the vehicle or left untreated. A similar pattern was seen for the challenge, with lower scores for the sunscreen formulation (approximating non-irritated skin). It was determined that the sunscreen formulation and its vehicle (minus active) did not induce photoallergic reactions under the conditions described above.

Clinical verification of skin tolerability, and evaluation of the cosmetic properties and acceptability of the substance; EVIC-CEBA Research and Experimental Labs.; Study No. Ie003/97.4404; M. Havet & P. Masson; 1998; GCP-yes (ref. 38).

It was stated that the test substance in the study was sun cream formula 427184, which was latter revealed to contain 10% G4375. This was stated to be a skin tolerability assessment of sun cream 427184 on 30-31 volunteers (aged 18 to 65 years). It was also noted that 40-45% of the subjects enrolled in the study presented with an allergic or atopic condition. Assessment of cosmetic properties and acceptability was conducted following application to the face under normal usage

conditions for approximately 21 days. Subjects in this study were classified as phototype I to IV according to the Fitzpatrick scale. Application of the test material involved gentle massage with the fingers, under normal conditions of use, on the face, twice daily, for 21 consecutive days. The use of other products of a similar purpose was banned during the course of the study. Principle criteria for evaluation of effect were the occurrence of erythema, oedema or vesicles, which were graded as being slight, moderate or severe. Background skin condition was assessed prior to commencement of the study, while the investigators carried out a final assessment on day 22. The subjects were required/invited to keep a daily diary identifying any effects they detected.

In the results it was stated that the group was composed of 24 females and 7 males, indicating a total of 31 subjects of which 45% had reactive facial skin associated with allergic or atopic conditions. There was no incidence of subject withdrawal during the course of the study. Therefore, the evaluation of cosmetics properties and acceptability was conducted on 31 subjects. A total of 5 subjects did not follow the protocol exactly with once a day use instead of twice and application to the neck as well as the face. Measurement of the returned samples of test material enabled the investigators to estimate the range of daily usage at 0.1 to 0.8 g/application; this compared with a theoretical/predicted value of 0.4 g/ application.

Investigator evaluation of the subject's skin at day 0 and day 22 was reported. The following summarises the findings:

Skin examination	Day 0	Day 22
Normal skin condition	21	25
Abnormal skin condition	10	6
Number of subjects examined during study	31	31

It is apparent from the data that the test material did not have an adverse on the condition of the skin, but appeared to enhance the condition in some individuals (subjects 7, 18, 27 and 30).

Assessment by the subjects revealed the perception of slight stinging around the nose and eyes (2 subjects), but this effect was transient. This response could have been linked to shaving. Another subject reported stinging and watering eyes on 2 days (days 1 and 3). Two subjects reported slight skin dryness on the face, above the eyebrows and on the nostrils. These reports were made by only a total of 4 subjects. Cosmetic properties such as pleasant texture, easy to apply, good penetration, pleasant perfume, non-greasy and non-sticky product, adequate skin moisturising, gives protection from the sun's rays and compatible with make-up were assessed. All properties except gives protection from the sun's rays and pleasant perfume scored above 61% of acceptance; gives protection from the sun's rays scored only 6% approval and pleasant perfume scored 39% approval.

Overall, the product sun cream appears to be acceptable as a cosmetic, but it has not gained acceptance as a sun filter according to an appraisal by the subjects. G4375, at 10%, did not appear to be performing a function as a sun filter based on subjective responses of the volunteers. This may have been due to limited (not appropriate for sun protection) application of the test material during the study.

Study of the tolerability and comedogenic potential of sun cream 427184 after repeated applications for twenty-nine days in thirty healthy volunteers of oily or mixed skin types prone to acne; Peritesco – Victor Hugo Clinic; Study No. PO354/D; S. Laquieze; 1998; GCP-yes (ref. 39).

This study examined the tolerability and comedogenic potential of sun cream 427184 (10% G4375) following twice-daily application for 29 days to subject (30) faces. Subjects were described as being aged between 19 and 61 with oily or mixed skin-type prone to acne. An estimated 0.25 g/application (0.5 g daily) of sun cream 427184 was delivered during the course of the study. Assessment of a series of parameters was conducted on days 1 and 29.

The incidence of skin stinging was slightly elevated (1 to 3 cases) over the course of the study. However, incidences of pruritus (1 to 0 cases), skin discomfort (4 to 2 cases) and tautness (4 to 1 cases) were all reduced over the course of the study. Physical signs of damage such as erythema (7 to 5 cases), skin dryness (9 to 3 cases) and skin roughness (2 to 1 cases) were generally reduced over the course of the study; skin desquamation went from 0 to 1 case. Overall, sun cream 427184 was well tolerated in individuals with oily skin and prone to acne.

Measurement of comedones and microcytes were used to evaluate the potential comedogenicity of sun cream 427184. Application of the test material resulted in alleviation of comedones in 12 cases and microcytes in 6 cases. There was only one individual that displayed an aggravation in microcyte intensity. It was noted that 17 subjects showed no change in comedone frequency, while 22 subjects showed no change in microcyst formation. These presented data indicated that 29 subjects (not 30 subjects) completed the study, which was verified in a volunteer identification table where subject 20 (SIMLU) was shown to have not returned on day 29 for final assessment.

Overall, the data showed that the sun cream was well tolerated by human skin.

File Note

4-Methylbenzylidene camphor

Introduction

This File Note is prepared in response to an email (dated 31/10/2005) from s47F who pointed out that the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) in EU is now asking the industry for more data on the safety of 4-methylbenzylidene camphor. s47F wanted to know whether TGA has any view on this issue.

Review by SCCP

In 2001, the SCCNFP evaluated the oestrogenic activity of a few sunscreen agents, including 4-methylbenzylidene camphor, and concluded that the organic UV-filters used in cosmetic sun-screen products, allowed in the EU market, have no estrogenic effects that could potentially affect human health. A review by the OTCMS, TGA in 2001 came to a similar conclusion regarding the oestrogenic potential of 4-methylbenzylidene camphor (Attachment 1).

The Scientific Committee on Consumer Products (SCCP) in EU received a submission from the industry ("Submission VII", appears to be from Merck KgaA, Germany) which reassessed the safety of 4-methylbenzylidene camphor concerning possible effects on the thyroid gland. The submission stated that the effects observed with 4-methylbenzylidene camphor in rats on the thyroid hormone profile and the thyroid morphological analysis had no relevance for man. Details regarding how this conclusion was reached were not provided. The SCCNFP assessed the submission and the available data (old and new), and submitted a report in May 2004 (Attachment 2).

Issues

The main issue is whether the use of 4-methylbenzylidene camphor as a sunscreen has the potential to affect the thyroid gland.

For the safety assessment, it is necessary to determine a no-effect level in toxicity studies (NOEL or NOAEL) so that exposure margin (safety margin; ratio or comparison of exposure at therapeutic level with that of the no-effect level) can be calculated.

Current regulation of 4-methylbenzylidene camphor in Europe

As in Australia, the maximum allowable concentration of 4-methylbenzylidene is 4% (as a UV filter) in sunscreen products.

Toxicity of 4-methylbenzylidene camphor

The following is a summary of toxic effects reported in the SCCP evaluation report:

Acute, short-term, subchronic studies: Acute toxicity is low. Short-term (up to 4 weeks) and sub-chronic (13 weeks) studies in rats suggested significant effects of 4-methylbenzylidene camphor on thyroid hormone metabolism as evidenced by changes in thyroid weight, levels of circulating thyroid hormones and histological evidence of thyroid stimulation (see below). In addition, interference with thyroid function may have affected other parameters (e.g. red blood cell turnover). All these studies were conducted prior to 1985 and all the study reports for the non-acute studies in rats appear to be in German.

Hypertrophy and hyperplasia of thyroid epithelium was observed at levels of 50 mg/kg bw/day and above. At the lowest level tested (25 mg/kg bw/day), no morphological thyroid effect was noted, but increases in serum T4 (thyroxine) were still observed. In addition, effects on red blood cell parameters were observed. A clear no-effect level was not obtained. Hence 25 mg/kg bw/day is a LOAEL rather than a NOAEL. No data were available on the long term consequences of prolonged thyroid stimulation.

Two short term studies (up to 3-weeks) were conducted in dogs but the studies could not establish a NOAEL since the studies were of short duration, the number of animals used in the study was low or there were no concurrent controls. A few changes were seen after treatment (trend for increased levels of thyroid hormones when compared to pre-treatment levels) but the study reports claimed that there were no treatment-related effects (eg. changes seen were within circadian variation). The SCCP could not use these studies to set a NOAEL.

Clinical studies: Two clinical studies (excluding one pilot study; one study complied with Good Clinical Practice) were reported in the SCCP. Both the studies were conducted with dermal application of the test substance. The GCP-compliant study used twice daily applications of 5 gram of a cream containing 6% 4-methylbenzylidene camphor (total dose = 600 mg/day). In these studies, there were no treatment-related effects, including effects on thyroid function. However, the SCCP criticised that the exposure surface was only 1200 cm^2 and commented that the exposure may not mimic the 'in use conditions'. However, please note that the actual dose applied was slightly higher than that expected during the normal use of 4-methylbenzylidene camphor in sunscreen products.

Reproductive and developmental toxicity: In a reproduction study in rats, the oral administration of levels up to 50 mg of 4-methylbenzylidene/kg bw/day did not affect reproductive function of female rats or the development of the offspring. A developmental toxicity study in rats revealed a NOAEL of 10 mg/kg bw/day with developmental effects seen at 30 and 100 mg/kg bw/day.

Skin irritation and sensitisation: Tests for skin irritation, sensitisation, photo-toxicity, photo-sensitisation and photo-contact allergy were negative. The SCCP report commented that the animal tests for sensitisation were unsatisfactory since Freund's complete adjuvant had not been used. However, the report noted that the compound very rarely caused contact allergy in man.

Genotoxicity: An Ames test and a chromosomal aberration test *in vitro* were negative. Tests for photomutagenicity in 2 strains *of S. typhimurium* and *E. coli* WP2, and tests for photo-clastogenicity were also negative.

Dermal absorption: According to the SCCNFP, the studies conducted on dermal absorption are old (1982-1984) and are not in accordance with modern guidelines, and the study results were difficult to interpret. In one clinical study, the absorption is estimated to be 0.9% but the SCCNFP estimated the value to be ~1.9%.

Opinion of the SCCP

The SCCP concluded that the reassessment of old and newly provided data indicated that the current use of 4-methylbenzylidene camphor in sunscreen products poses a reason for concern. It also stated that although the changes in thyroid hormone profile and thyroid morphological analysis in rats were difficult to interpret with the data available, increased TSH in combination with elevated T3 or T4 (triiodothyronine and thyroxine, respectively), enlarged thyroids and thyroid proliferation suggested a major interference of 4-methylbenzylidene camphor in thyroid hormone metabolism. According to the SCCP, risk assessment is further hampered by the lack of adequate data on dermal penetration and the fact that 25 mg/kg body weight/day is a LOAEL rather than a NOAEL in rats.

The SCCP requires the following additional information as a matter of urgency for a better evaluation of the potential effects of 4-methylbenzylidene camphor:

- complete physico-chemical data;
- a dermal penetration study according to current guidelines, including the study of the different factors affecting the quantitative outcomes of the results;
- a clear NOAEL obtained in a relevant species;
- exposure data on other uses and on oral intake.

Conclusions and recommendation

It is clear from the SCCP that a no-effect level cannot be determined from the available studies. However, for estimating the margin of exposure (to assess the safety) of 4-methylbenzylidene camphor, the safety factor used in the estimate can be adjusted: a higher factor (eg. 500), instead of the usual 100, can be used if the margin of exposure is calculated from the LOAEL rather than from the no-effect level.

A major problem is the absence of reliable data to estimate the dermal and oral absorption of 4-methylbenzylidene camphor: oral absorption data is needed to interpret the data of rat studies which were conducted with oral administration of the test substance; and dermal absorption data is needed to estimate exposure following use of 4-methylbenzylidene camphor containing topical products.

A human study (n = 24/sex) has been conducted following topical application of 4-methylbenzylidene camphor and using Good Clinical Practice guidelines. There were no

treatment-related effects (including effects on thyroid hormone levels) suggesting that there is no significant cause for concern. However, the study was of short duration (treatment for 2 weeks; parameters monitored up to a week after the treatment period) and hence long term effects of 4-methylbenzylidene camphor are not known. The study report argued that since the thyroid related hormone with the longest half life in the serum, thyroxine, had a half life of 7 days, a 14 day study would pick up any variations in the levels of thyroid related hormones. The SCCP commented that the exposure surface of the test substance was only 1200 cm^2 but it is to be noted that the study has used slightly higher amounts of the test substance (total amount/day) that would be normally used in sunscreen products.

4-methylbenzylidene camphor has been used in a number of products for some years, and it appears that there are no major adverse effects reported for the use of 4-methyl-benzylidene camphor in sunscreen products. A clinical study (but a short-term study) which followed GCP conditions did not find any significant adverse effects on the thyroid function. Based on these, there is no cause for urgent action and hence it is recommended that we wait for the outcome of the SCCP's evaluation of 4-methylbenzylidene camphor.

s22 [redacted]
Senior Toxicologist
OTC Medicines Evaluation Section
11 November 2005

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers. It appears that the SCCNFP was a former committee of the EC and was replaced in 2004 with the Scientific Committee on Consumer Products (SCCP).

Appears to be from Merck KgaA, Germany as per another communication from the SCCP.

The scientific committee on cosmetic products and non-food products intended for consumers. Opinion concerning 4-methylbenzylidene camphor. Colipa nº S60. SCCNFP/0779/04. Adopted by the SCCNFP during the 28th plenary meeting of 25 May 2004.

For safety assessment, some prefer to use the NOEL (no observable effect level: level showing no treatment-related effect at all) whereas others use the NOAEL (no observable adverse effect level; some changes could occur at this level, but the changes are not considered biologically significant).

LOAEL is the lowest observable adverse effect level (lowest dose which shows adverse effects) while NOAEL is the no observable adverse effect level.

Assuming a product containing up to 4% of 4-methylbenzylidene camphor is applied on the skin at a maximum amount of 18 g. ie. 18,000 mg product x 4% 4-methylbenzylidene camphor = 720 mg 4-methylbenzylidene camphor per day.

OTC MEDICINES SECTION – TGA

SAFETY EVALUATION OF NEW SUNSCREEN ACTIVE

POLYSILICONE-15

Sponsor:	s47 [redacted]
Consultant:	s47F [redacted] & Associates Pty Ltd
Proposed AAN:	Polysilicone-15
Trade names:	PARSOL SLX
Lab. Codes:	RO84-5690/001 & Giv/Ro 84-5690
INCI name:	Polysilicone-15
Chemical name:	Dimethicodiethylbenzalmalonate
Empirical formula:	$C_{196}H_{490}O_{84}Si_{65}$
CAS No:	207574-74-1
Molecular weight:	5987 (of main homologue)
Weight average:	23354
Chemical class:	Polysiloxane polymer (polydimethylsiloxanes – PDMS)
Appearance:	Clear slightly yellow viscous liquid
Melting point and /or boiling point:	$>210^0$C, under 101 hPa
Freezing point:	No crystallisation observed down to 223^0K
Solubility:	Very low in water, <0.1 mg/L after 24 hours
UV spectrum:	Absorbance maximum at 312 nm
Proposed use:	UVB filter in listed suncare products at a concentration of 10%.

Structure:

SUMMARY OF TOXICOLOGICAL FINDINGS

Absorption data single dermal dose in rats (*in vivo & in vitro*)	0-0.2% (2%)
In vitro percutaneous absorption assay with human skin	0.061% (0.476%)
Absorption data single oral dose in rats (*in vivo*)	≤1.5% (app. 20%)
Acute oral toxicity in rats	>2000 mg/kg
Acute dermal toxicity in rats	>2000 mg/kg
Primary eye irritation in rabbits	non-irritant
Primary skin irritation in rabbits	non-irritant
Skin sensitisation (max. test) in guinea pigs	non-sensitiser
Human RIPT for contact sensitisation	negative
Photosensitisation assay in humans	negative
Photo-toxicity in guinea pigs	negative
Photo-allergenicity in guinea pigs	negative
13 weeks oral toxicity study in rats	NOEL 1g/kg/day
Oestrogenic activity in MCF-7 cells (*in vitro*)	negative
Developmental toxicity study in rats (oral dosing)	NOEL 1 g/kg/day
Genotoxicity Ames assay *in vitro*	negative
Mouse lymphoma cell (ML/TK) test *in vitro*	negative
V79 Chinese hamster lung cell chromosomal aberration assay *in vitro*	negative
Photomutagenicity assay using *Saccharomyces cerevisae in vitro*	negative
Photoclastogenicity assay in Chinese hamster cells *in vitro*	negative
Carcinogenicity potential	no bioassay

Introduction

Polysilicone-15 is a new UV filter (absorbance max. 312nm; UVB range) for use in listed sunscreen products. It is proposed that it could be used in primary and secondary sunscreen (also in cosmetics) to protect against damage caused by excessive exposure to sunlight. The sponsor noted that polysilicone-15 consists of a cinnamate bonded to a polymeric silicone. It was stated that statistically, 4 UV absorbing units are bonded to every 60 units of the polymeric chain. Furthermore, properties of polysilicone-15 suggest that it remains in the stratum corneum, offering UV protection and minimising skin penetration. Assay procedures for the identification of polysilicone-15 were included in the data provided.

International status

Polysilicone-15 was included on the European Cosmetic Directive (76/768/EEC) Annex VII Part 1 in April 2002, which is the list of permitted UV filters. Approval was based on an assessment of a safety dossier by the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) of the European Commission. It was noted that polysilicone-15 (up to 10%) is currently in 3 sunscreen products on the European market.

Polysilicone-15 is registered in Taiwan with a maximum authorised concentration of 10%.

International regulatory comment

The SCCNFP was of the opinion that polysilicone-15 is safe for use in cosmetic products as a UV light absorber at a maximum concentration of 10%. They concluded that, “the substance appears not to be an irritant to the skin or mucous membranes, and not to induce sensitisation or photo-sensitisation. In is not mutagenic or clastogenic, whether in the presence or absence of ultraviolet radiation. It has low acute toxicity by dermal and oral routes of administration. It does not induce photo-toxicity. A well conducted oral rat study gave a NOAEL of 1000 mg/kg/day. Percutaneous absorption was studied using pig and rat skin *in vitro*. There is some difficulty in assessing the results of this experiment; if the amounts in the stratum corneum are taken to be absorbed the absorption may be as high as 2%, but no active ingredient was found in the receptor fluid”.

The SCCNFP calculated a safety margin based on maximum amount of ingredient applied (based on 18 mL at 10%) of 1800 mg, in a 60 kg person, with a maximum absorption through the skin of 2% (1800 x 2% = 36 mg/60 kg) leading to a figure of 0.6 mg/kg related to dermal absorption per body weight unit.

They then took the NOAEL (1000 mg/kg) from the rat study and divided it by the amount likely to be absorbed through the skin (exposure) of 0.6 mg/kg and came up with a margin of safety of 1667.

s47 [redacted]

Interaction with other UV filters

There did not appear to be any data on the possible interaction of polysilicone-15 with other UV filters likely to be used to formulate sunscreen products. Information on the stability (to hydrolysis) of Parsol SLX (5% formulation) was presented in graphical form, which showed no variation in Parsol SLX (100% recovery in all instances) under conditions of changing pH (5-8) and temperature over periods of 6 (80ºC) and 48 (60ºC) hours, and at 43ºC for 3 months. Additional information on photo-stability showed thin film layers of sunscreen formulations (includes Parsol SLX) irradiated with a UV dose equivalent to 10 MED (minimal erythemal dose) delivered over a 5 hour period did not degrade. The sunscreen formulations (including Parsol SLX) were tested according to prescribed application rates of sunscreens to human skin. The application rate, as used in SPF testing, was 2 mg/cm^2 onto the glass plate prior to UV irradiation. The concentration of Parsol SLX used in this test was not recorded.

Included in a recent submission was a letter describing the use of PARSOL SLX in combination with a number of UV absorbers available on the market, which included UV absorbers/filters used in the sponsors ([redacted]) own range of sun-care products. The following UV absorbers have been combined with PARSOL SLX without any compatibility problems. The list includes ethylhexyl methoxycinnamate, butylmethoxydibenzoyl methane, phenybenzimidazole sulfonic acid, octocrylene, 4-methylbenzylidene camphor, titanium dioxide, ethylhexyl triazone and Tinosorb M. The definition of compatible in this case appears to refer to stability, since it was stated that, “there were no antagonistic effect with regard to stability”.

Adverse effects

Included in the submission was an assessment using a computer program (Derek for Windows) that examined the potential toxicity of the test material compared with a positive control (8-methoxypsoralen; known photo-carcinogen and photo-sensitiser). It was noted that the analysis revealed no identifiable risk from the test material when compared to the positive control. A statement was included indicating there are no known, or potential, adverse effects from the use of polysilicone-15.

A metabolic fate profile computer program (Meteor) was used to predict likely metabolites generated from the parent compound. An extension of this analysis (structure-activity relationships) included the assessment of these metabolites using Derek (toxicity screening computer program), which is used to predict the presence of hazard alerts associated with chemicals. It was stated that, “the predicted metabolites did not generate a list of alerts”, which suggests a lack of potential toxicity.

SUMMARY/ASSESSMENT

The data package contained studies that were conducted under conditions of good laboratory/clinical practice (included quality assurance statements). Studies submitted included dermal absorption through skin from humans, rats and domestic pigs (*in vitro* percutaneous) and rats (*in vivo*), oral absorption in the rat (*in vivo*) local tolerance (skin and eye irritation in rabbits), sensitisation and photo-sensitisation (animals and humans), acute toxicity in rats (dermal and oral), repeat-dose oral toxicity in rats (13 weeks), reproductive toxicity (developmental study in rats and *in vitro* oestrogenic activity assay), and genotoxicity and photo-genotoxicity (all *in vitro*) assays. There were no data on *in vivo* genotoxicity endpoints.

Pharmaco/toxico-kinetics

In vitro studies on percutaneous absorption of polysilicone-15 in humans, rats and domestic pigs and an *in vivo* dermal absorption study in rats were provided in support of this application. Data were also submitted on the absorption of polysilicone-15 via the oral route in rats, which is considered necessary for a thorough assessment of exposure associated with oral repeat dose and reproductive toxicity studies in rats.

Specific data on possible/potential metabolic conversion of polysilicone-15 were absent. A metabolic fate computer program (Meteor) was used to predict likely metabolites resulting from metabolism of polysilicone-15 *in vivo*. These metabolites were assessed for potential risk/hazard using a toxicity computer program (Derek). It was indicated that the likely metabolites of polysilicone-15 breakdown would not be considered a risk/hazard.

Both the *in vitro* (human, rat, pig skin) and *in vivo* (rat) dermal absorption assays produced results suggesting negligible (0.061%) levels of polysilicone-15 were found in receptor fluid (represents absorption across skin) or plasma following up to 24 hours (range 16-24 h) contact with the skin. Estimations of potential systemic exposure were related to amounts of polysilicone-15 found in tissues (stratum corneum) that may act as a reservoir for absorption of the test material over an extended period. In the rat study for dermal absorption of labelled polysilicone-15 *in vivo*, 0.2% of the administered label was detected in the urine, which may indicate that this amount of the test material was absorbed.

Calculation of safety margins following application of polysilicone-15 is present in the preceding International Regulatory comment section. The SCCNFP came up with a figure of 1666, s47 [redacted]. The difference in these results appears to be related to the values (2% vs 0.5%) used for *in vitro* percutaneous absorption. The 0.5% (used by product sponsor) value comes from the human *in vitro* percutaneous study, which does not appear to have been seen by the SCCNFP. A value of 2% used by the SCCNFP was taken from the *in vitro* rat and pig studies and they suggest that, "if the amounts in the stratum corneum are taken to be absorbed the absorption may be as high as 2%, but no active ingredient was found in the receptor fluid". Therefore, the time and amount in contact with the stratum corneum (also its turnover?) that could lead to penetration (possible absorbed) would be important factors regarding potential systemic exposure. The SCCNFP value is stated to be an upper limit of exposure (expected maximum possible), which is likely to be less in reality based on expected length of exposure to the sunscreen (16 hours in assay compared to use in sun

during day) and lack of external influences such as abrasive forces (towels, sand, water) that are not present in the *in vitro* assay.

Use of the dermal absorption value (plasma concentration) from an animal (rat as opposed to human value) in the calculation of safety margin may further complicate the measurement. This belief is based on reported work that showed rats have typically 2- to 5-fold higher percutaneous absorption values when compared to humans (from Skin Barrier, Principles of Percutaneous Absorption, H. Schaefer & T. Redelmeier, Kargar Press, p120, 1996). However, it would appear that the SCCNFP only had animal data to refer to for their calculation. Furthermore, estimates of safety margins should be based on relative exposure (using AUC data) between dermal and oral routes to provide an appropriate link to the oral toxicity studies in rats and generate a NOEL used in safety calculations. Therefore, the safety margins estimated by the sponsor and the SCCNFP provide limited reassurance.

Data examining plasma concentrations of polysilicone-15, given by the oral route (2 and 1000 mg/kg/day) in rats, enabled a limited relative assessment of exposure in the repeat dose and reproductive oral toxicity studies to be conducted. Doses used during analysis of oral absorption were levels used in a pilot kinetic study and the highest level used in the repeat-dose toxicity studies. The data analysis presented plasma concentrations of the labelled test material, which is not the most appropriate measurement of systemic exposure (should be AUC data). Following oral administration of 2 or 1000 mg/kg/day ^{14}C-PARSOL SLX, plasma concentrations of radioactivity were generally only measurable in animals given 1000 mg/kg/day. Urinary recovery data indicated that approximately 1.5% of the 2 mg/kg/day dose and 0.7% of the 1000 mg/kg/day was absorbed. Excretion data for removal in the faeces showed a range of 61.5% to 75.0% recovered, which may mean greater than 20% radioactivity (PARSOL SLX) was retained in the carcass?

It could be assumed that the amount of test material/radioactivity in the carcass (following oral dosing) is available to the system in a similar manner to the amount of test material available in epidermis (following dermal application) as proposed in the SCCNFP safety margin calculation. Therefore, oral dosing could result in appreciably greater (20% in carcass vs 2% in epidermis) exposure to the test material than is the case following dermal application. Data from the oral toxicity studies in animal, indicating no toxicity at the highest dose, would appear to be based on exposure greater than is likely to occur following dermal application. That reasonable exposure occurred in animals (rats) following oral dosing is supported by the apparent changes identified as resulting from metabolic adaptation to a xenobiotic (test material).

The human *in vitro* percutaneous assay showed negligible (0.061% in assay with human skin) levels of polysilicone-15 were found in receptor fluid (represents concentration of applied dose absorbed across skin) or plasma (rats) following up to 24 hours (range 16-24 h) contact with the skin. Urinary concentrations (marker of exposure) following oral dosing in rats were in the range of 0.7%-1.5%, which is significantly greater (>11 times) than concentration detected in receptor fluid following dermal application of the test material.

Interaction with other UV filters

There did not appear to be any information of the possible interaction of polysilicone-15 with other UV filters likely to be used to formulate sunscreen products. Information on the stability of polysilicone-15 was presented, but this was in isolation from other UV filters.

Polysilicone-15 was shown to be resistant to degradation under extremes of pH and temperature over periods of up to 3 months.

Local tolerance

Polysilicone-15 was classified as a non-irritating agent to the rabbit eye, with effects (erythema & chemosis) limited to the conjunctivae for 24 hours after application (not seen at 48 or 72 hours). The test material was not washed out of the eye after instillation into the conjunctivae. In a skin irritation study in rabbits, minimal erythema was observed after removal of a semi-occlusive dressing 24 hours after application of the test material. Based on an irritation index of 0.11 polysilicone-15 was determined not to be a primary skin irritant.

Potential contact skin sensitisation activity of polysilicone-15 was assessed initially in a maximisation assay in guinea pigs. This study used appropriate numbers of animals and procedure, with the polysilicone-15 tested at a 100% concentration during induction and over a number of concentrations (5, 10, 30 & 100%) at challenge. Comparison with relevant controls indicated that polysilicone-15 did not induce skin hypersensitivity in guinea pigs at induction and challenge concentrations up to 100%.

A repeated-insult patch test was conducted using a 10% polysilicone-15 formulation on a panel of 103 human subjects. A total of 92 subjects completed the study in a satisfactory manner, with the reasons for omission/drop-out of the 11 subjects unrelated to exposure to polysilicone-15. Polysilicone-15 did not cause skin irritation (not seen at any induction time point) or sensitisation (challenge negative) in humans.

Photo-toxicity and -allergenicity assays were conducted using guinea pigs with concentrations of polysilicone-15 up to 100%. In these assays, polysilicone-15 was negative for adverse effects and was determined not to be phototoxic or a photoallergen in the guinea pig.

A photosensitisation assay was conducted using a panel of 30 human subjects and a concentration of 10% polysilicone-15. Polysilicone-15 did not cause a reaction at any time during the induction process or at challenge during this study. Polysilicone-15 was not a photosensitiser in this assay. The conduct of this study was acceptable, although the size (n=30) of the panel used to test polysilicone-15 was on the small side. Data for all 30 subjects were represented in the tables of results.

Overall, the likelihood of polysilicone-15 having adverse local (and immunological) effects on normal human skin appears negligible to low. The subject size (n=30) of the photo-sensitisation assay was not large, but an absence of positive reaction across all studies (humans and animals) is reassuring. Furthermore, negative results for skin sensitisation and photo-sensitisation studies (humans and guinea pig) indicate negligible interaction of the test material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and, potentially, to DNA.

Acute/repeat dose toxicity

Acute oral and dermal toxicity studies in rats showed that polysilicone-15 has low acute toxicity (>2 g/kg). In these studies, there was no evidence of toxicity with no clinical signs of

toxicity, no departure from normal body weight development and no unusual lesions at necropsy.

A thirteen week repeat dose oral toxicity study in rats used doses of up to 1000 mg/kg/day delivered by gavage. A total of 2 deaths (control & HD animal) occurred during the study, which were not linked to treatment with polysilicone-15. An observation during organ weight analysis was minor differences in relative liver weight (increased in HD males only), as well as some variations in clinical chemistry (slightly elevated bilirubin, significant at high dose only) findings across the groups. The increase in relative (to body weight) liver weight was 16% in the HD male group, while the LD and MD groups were slightly above the control value. Analysis of the liver weight after the drug-free recovery period showed a value marginally (6%) above the control value, indicating a change toward control levels. Histopathological analysis did not reveal any adverse changes in the liver at any dose level compared to the control group. The change in liver weight appears to be of no toxicological concern since it was not accompanied by structural changes. It was proposed that the increase in liver weight possibly represented an increased demand for liver function to eliminate the test substance, which appears to be a reasonable conclusion.

Acknowledging a potential stimulation of liver metabolic function as a reaction triggered by polysilicone-15 would suggest that oral dosing leads to some degree of systemic exposure. The extent of this exposure was estimated to be up to approximately 1.5% (based on plasma concentration data) through kinetic determination of oral absorption in the rat. This possibility is further supported by changes in plasma bilirubin that are consistent with metabolism of xenobiotic and a sign of metabolic adaptation following systemic exposure to the test material.

If sufficient exposure to polysilicone-15 occurs to justify an interpretation of metabolic adaptation it could be accepted that no relevant toxicity was generated by oral exposure to polysilicone-15 up to 1000 mg/kg/day. However, this highlights a further issue (in toxicological terms), since no toxicity was seen at the highest dose used, and therefore the study could be considered inadequate since the dose was not pushed to a level to precipitate toxicity. A lack of toxicity at 1000 mg/kg/day PO could be due to low intrinsic toxicity of the test material or due to poor absorption from the GIT. The low (approx. 1.5%) level of potential absorption (and systemic exposure) seen following oral dosing could be an underestimation, with the recovery data (up to 75%) possibly supporting this conclusion. Release from the carcass/tissues over the duration of the study (and beyond) may have occurred or have the potential to occur. This highlights the issue of measuring systemic exposure using AUC data and not plasma (Cmax) data.

Reproductive toxicity/hormonal activity

The sponsor submitted two studies addressing potential reproductive aspects of the dermal use of polysilicone-15 in humans. The first study was a transactivation assay in transfected MCF-7 cells to determine the oestrogenic potency of polysilicone-15. The system used in the study was transfected MCF-7 cells, constitutively expressing both oestrogen receptor isoforms ER and ER stably transfected with a reporter gene under control of the oestrogen regulatory element (gene promoter). In this model, oestradiol induces a marker (luciferase) in a dose-dependent manner at sub-nanomolar concentrations, with maximum levels of this marker reached by 24 hours after incubation of cells with oestradiol. It was shown that the positive control oestradiol stimulated significant dose-dependent increases in oestrogenic

activity, which was blocked by oestrogen antagonists. Polysilicone-15 was negative in this assay showing it did not possess the capacity to induce oestrogenic activity in transfected MCF-7 cells *in vitro*.

In an *in vivo* segment II reproductive bioassay, mated female rats were exposed to polysilicone-15 administered orally at doses up to 1000 mg/kg/day during the period of organogenesis (gestation days 6 through 20). Results indicated that exposure to the test material during this sensitive development period had no adverse effects on reproductive parameters, maternal health or foetal development. Fewer mated females were pregnant at the higher dose levels (300 & 1000 mg/kg/day) compared to the control and LD (100 mg/kg/day) groups, but this is not due to an effect of treatment since exposure to the test material did not occur until day 6 of gestation. Overall, the NOEL for this study was estimated to be 1000 mg/kg/day PO.

Assessment of this study (rat development) is helped by oral toxicokinetic data in the same species (rat). It is likely that the anticipated (based on oral kinetic study in non-pregnant rats) plasma levels of the test material would result in exposure above what would be achieved following dermal administration. Assessment of potential oestrogenic interaction was limited to an *in vitro* assay using transfected cells. There was no *in vivo* assay (eg rat uterotrophic) to confirm the findings seen in transfected cells.

Assessment of the reproductive toxicity of polysilicone-15 is very limited with only a segment II developmental study (in rat) and an *in vitro* oestrogenic activity assay submitted for evaluation.

Genotoxicity

A series of *in vitro* assays were submitted in support of this application. Testing included a reverse mutation assay (Ames test), a chromosomal aberration assay in Chinese hamster V79 cells, a mouse lymphoma cell mutation test (ML/TK), a photo-mutagenicity assay in yeast *Saccharomyces cerevisiae* D7 and a photo-clastogenic assay in cultured Chinese hamster cells (±S9).

End points in these assays included reverse/forward mutation and chromosomal aberrations. All assays (including those with UV exposure) produced negative results indicating that polysilicone-15 was not genotoxic *in vitro* in the presence or absence of UV radiation.

The validity of these tests could be questioned on the grounds that polysilicone-15 (mean MW 23354) may not have been able to penetrate cells to interact with genetic material. Reference molecules cited in past assessments as being large but are able to penetrate include some antibiotics, however, these antibiotics (eg vancomycin, MW 1449) are a fraction of the size of polysilicone-15. Therefore, it is valid to question the value of these *in vitro* assays when the size of poysilicone-15 is taken into account. It should also be noted that cell penetration is based on more factors than just size and an exact understanding of whether polysilicone-15 penetrates into bacterial and mammalian cells (*in vitro* assays) appears unavailable.

The inclusion of an *in vivo* assay (mouse micronucleus) would greatly enhance the credibility of the genotoxicity data package.

Carcinogenicity

A carcinogenicity study was not submitted in the application. The following is a response by the sponsor addressing the issue of potential carcinogenicity of polysilicone-15.

No long-term carcinogenicity study has been conducted on polysilicone-15. This was considered to be unnecessary in view of the following:

1. Polysilicone-15 is a polymer, the main homologue of which has a molecular weight of nearly 6000. The sponsor noted this is more than 11 times the molecular weight that is generally regarded as the upper limit for absorption through the stratum corneum (Bos & Meinardi; Exp. Dermatol; Jun 2000; 9(3), 165-9).

2. Polysilicone-15 was found not to be mutagenic in a series of *in vitro* assays that examined clastogenic (chromosomal aberrations) and point mutation end points. It was negative in photo-assays for clastogenic and aneuploidogenic activity.

3. An assessment of risk for carcinogenicity, genotoxicity, thyroid toxicity and other end points using DEREK for windows across a range of mammalian and other organisms produced no safety alerts.

4. There are no similar molecules in the NTP list of carcinogens or in the most recent IARC list of monographs.

5. A comparison was provided for polysilicone-15 with other silicones. It was noted that a related compound polydimethylsiloxane (+ related molecules) had been shown to have very low toxicity in dietary (13 weeks) and developmental toxicity studies in rats and mice. An assessment (CIR report) of dimethicone noted that it was negative in oral and dermal genotoxicity assays in mice.

6. Polysilicone-15 contains cinnamate groups that are found in a number of UV filters accepted for use in sunscreens in the USA, EU, Australia and numerous other countries. These UV filters have a history of safe use in these countries and none of these compounds appear in any published list of carcinogens.

7. Polysilicone-15 was shown not to be an irritant to skin at 10% concentration in a human repeated-insult patch test (HRIPT).

8. Polysilicone-15 was shown not to be an irritant to skin at 100% concentration when applied to rabbits or in a HRIPT (10% concentration).

9. Polysiliocne-15 was not an ocular irritant at a concentration of 100% in the rabbit eye.

10. Polysilicone-15 was not a skin sensitiser in the guinea pig under conditions of increased reactivity (maximisation assay). It was not a skin sensitiser in a HRIPT.

11. Polysilicone-15 was not a photoallergen in a guinea pig assay or in a human assay.

The toxicity profile of polysilicone-15 suggests it would have a low potential to be a carcinogen. Polysilicone-15 was not genotoxic in a limited (no *in vivo* assay) series of studies including photomutagenicity assays (*in vitro*). It would appear that no direct data on the metabolism (ADME study) are available for polysilicone-15 so the potential for the formation of active metabolites is not accurately known. Computer modelling programs for metabolism (Meteor) and toxicity (Derek) of potential metabolites provided supporting information that suggested likely metabolites would not present a health hazard.

In the 13 weeks repeat dose toxicity study in rats, the findings indicated no apparent histopathological evidence of neoplastic changes, although this period of exposure is extremely short and unlikely to induce neoplastic changes. In the oral developmental study in rats, there was no evidence of adverse effects on rapidly differentiating tissue following exposure during organogenesis.

Polysilicone-15 was not a skin irritant or skin sensitiser (or photosensitiser), which would indicate negligible interaction of the test material with sensitising molecules (are either electrophilic or form electrophilic metabolites) that result in adducts to proteins and, potentially, to DNA.

A non-genotoxic mechanism for carcinogenesis involving chronic skin irritation and inflammation could be relevant for topically applied substances such as UV filters in sunscreens. However, data are available from skin irritation studies (in rabbits and humans), which indicate polysilicone-15 was not a primary skin irritant. There was no repeat-dose dermal toxicity study submitted in this application, which does not allow an evaluation of the effects of repeated dermal exposure.

RECOMMENDATION

The sponsor has submitted a package of data containing studies on polysilicone-15 that conform to GLP standards and OECD or ICH guidelines regarding conduct of studies (includes QA statement). Polysilicone-15 is a UV filter that has been on the accepted list of UV filters in the EU since April 2002 at a concentration of up to 10% in products.

There was no definable toxicity detected in animal studies (13 weeks repeat-dose oral and oral developmental in rats) at doses up to 1000 mg/kg/day. Changes associated with liver (increase in liver weight and plasma bilirubin) function observed in the 13 weeks oral toxicity study in rats were said to be related to metabolic adaptation due to the presence of a xenobiotic (polysilicone-15), since no related histopathology (or additional clinical chemistry changes) were detectable. Results from these studies lead to an estimate for the NOEL of 1000 mg/kg/day. Studies in animals and humans demonstrated that polysilicone-15 was not a skin irritant or sensitising agent, and it was not a phototoxic or photosensitising agent following limited dermal exposure (no long-term dermal toxicity studies provided).

A series of *in vitro* genotoxicity studies (and photomutagenicity assays) examining potential adverse effects of polysilicone-15 on structural genetic material was negative. No *in vivo* genotoxicity studies were presented in this application.

Pharmacokinetic data submitted in support of this application included *in vitro* and *in vivo* dermal absorption assays for rat, pig and humans; these showed very low dermal absorption. There were oral toxicokinetic data in the species (rat) studied in the toxicity assays. Toxicokinetic data (based on plasma concentrations, not AUC analysis) indicated that oral toxicity studies (NEL 1000 mg/kg/day) were likely to achieve greater systemic exposure than occurred following dermal application of the test material.

Justification for an absence of a dermal carcinogenicity bioassay was based on the toxicity profile (absence of toxicity) and no structural alert features triggered by the molecule. All genotoxicity assays were conducted *in vitro*, which has been seen in past applications as not covering appropriate genetic end points. An *in vivo* genotoxicity study (eg. mouse micronucleus) has been identified as establishing a greater degree of assurance and support an absence of concern. No such assay was provided for this application.

Based on previous opinion regarding genotoxicity data and justification for not conducting a carcinogenicity bioassay, an *in vivo* genotoxicity assay would greatly enhance the credibility of the genotoxicity data package.

The advice/opinion of the committee is requested.

EVALUATION OF SUBMITTED TOXICITY DATA

TOXICOKINETIC/PHARMACOKINETIC DATA

> *In vitro* percutaneous absorption of Parsol SLX from a 10% o/w formulation through human epidermis; s47F ., UK; study no. JV1795; s47F ; 2004; GLP/QA-yes.

The absorption of Parsol SLX from an o/w formulation across human epidermis (includes stratum corneum) *in vitro* was examined. The test formulation was applied to prepared human epidermis (total of 10 skin samples form 4 individuals) that was positioned across a chamber that contained a receptor fluid. The test material was applied at a nominal rate of 2mg/cm^2 (equivalent to 208.4µg ParsolSLX/cm^2) and left in contact (unoccluded) for a period of 24 hours. This exposure regimen was meant to simulate normal usage patterns for this type of product. At the end of the exposure period, the distribution of Parsol SLX in the test system (receptor fluid) was determined, as well as its permeation into the epidermis (using skin stripping techniques).

Data presented showed a mean penetration rate for Parsol SLX of 0.005µg/cm^2/h over the 24 hours of epidermal contact. A breakdown of the rates over 24 hours showed that from 0-6 hours it was 0.004µg/cm^2/h, while from 6-24 hours it was 0.006µg/cm^2/h. An average of 0.061% of the applied dose was found to have penetrated the epidermis at the end of the 24 hour exposure period. Skin stripping showed distribution in the epidermis could be divided into 0.730% retained in the stratum corneum and an additional 0.415% in remaining structures. A figure of 0.476% (0.415% + 0.061%) was identified as the mean total amount of Parsol SLX absorbed (amount in epidermis + penetrated amount) in this assay.

Recovery of the originally applied dose was found to be very good (102%), which was made up of the amount in the receptor fluid, the amount in the epidermis and the amount removed from the epidermis after the 24 hours exposure period.

It was concluded that the penetration of Parsol SLX into the skin was extremely slow, with the majority of the applied dose likely to be removed by cleansing of the surface or natural desquamation. It was suggested that the amount of Parsol SLX absorbed from this particular o/w formulation would be minimal (0.476%). It was also noted that only 0.061% had penetrated into the receptor fluid across the epidermis over the 24 hours exposure period.

> Percutaneous absorption of Parsol SLX; identified as progress report for Givaudan Roure Corp. research; BCR no. 151572; s47F ; March 1996; stated to be conducted according to GLP, but no QA.

In this study, the apparent focus appeared to be on establishing whether Parsol SLX remained on the skin surface (minimal absorption), which is a necessary condition for a sunscreen (UV filter) to be effective; hence the study looked at skin penetration as an efficacy issue and not so much as a safety issue. It noted that percutaneous absorption of a compound through the skin is not a fixed value, “but depends on many factors such as vehicle, concentration, exposure time, number of applications, skin type, skin surface condition, etc”.

In this study the absorption of Parsol SLX through/into hairless rat and domestic pig skin was assessed and compared with a “bench mark” molecule Parsol MCX (octyl p-methoxy

cinnamate). This was an *in vitro* assessment of skin penetration using a modified Franz cell and prepared rat and domestic pig skin. Parameters associated with substance application were: application area 5 cm^2, application amount of 2 mg/cm^2, chamber continually stirred and kept at 32^0C, however, the composition of the receptor (10 mL volume) fluid was not clearly stated, but a diagram of the set-up had a caption indicating receptor phase was NaCl (9g/L) or PBS + Volpo N20. The test substance was at a concentration of 5% in an o/w formulation. Included in this caption was a statement that penetration was 1 to 16 hours (exposure of skin to test material).

Rat skin: the skin used in this assay was described as "naked" following removal of the stratum corneum through the use of adhesive tape; a process called stripping (mean 15 repetitions to achieve naked skin). The description of preparation for the skin samples used in the assay stated that the stratum corneum was removed prior to application of the test formulation. However, tabulated (histogram) data indicated measurements of test material in the stratum corneum were carried out and recorded. It would appear that this procedure was also carried out using intact skin.

The prepared skin was placed across the top of the receptor fluid and the test substance applied to a marked area of 5 cm^2 in the middle of skin. Total test substance applied was 10 mg (5cm^2 x 2mg/cm^2). After the designated exposure period (16 hours) the area of skin exposed to the test material was analysed for the presence of Parsol SLX. A table of collated penetration data indicated that for Parsol SLX 98.2%, 1.4%, 0.4% and 0% of the applied dose was found on the skin surface, stratum corneum, in stripped skin and the receptor fluid, respectively. Figures for Parsol MCX were 40.1%, 12.7%, 34.0% and 13.2% of the applied dose found on the skin surface, stratum corneum, in stripped skin and the receptor fluid, respectively.

Pig skin: data for this section of the study was presented in a similar manner to the rat section, but included a comparison of absorption using a variety of vehicles (o/w cream or lotion and petrolatum). A table of collated penetration data indicated that for Parsol SLX 97.7%, 2.1%, 0.2% and 0% of the applied dose was found on the skin surface, stratum corneum, in stripped skin and the receptor fluid, respectively. Figures for Parsol MCX were 94.0%, 4.0%, 1.7% and 0.3% of the applied dose found on the skin surface, stratum corneum, in stripped skin and the receptor fluid, respectively. A comparison of the effect of different vehicles showed relatively small variations in penetration of either Parsol SLX or Parsol MCX in skin surface (range 90.8-98.1%), stratum corneum (range 2.1-4.0%), in stripped skin (range 0.2-4.4%) and the receptor fluid (range 0-1.0%). These data suggests that the majority of test material stays in the stratum corneum.

A comprehensive analysis of this study is limited by a lack of detail regarding the number of experiments performed to generate each data point and a lack of description of the statistical approach used to produce the data. However, it was evident that Parsol SLX did not readily penetrate either rat or pig skin *in vitro* based on the data for the amount detected (zero) in the receptor fluid after a 16 hours exposure period.

> Pharmacokinetic study of radiolabelled Parsol SLX following dermal application to rats; s47F ., USA; study no. 968-002; s47F ; November 2003; GLP/QA-yes.

This study examined the plasma pharmacokinetics of ^{14}C-Parsol SLX following a single application of 2 mg/kg (application volume 10 µL/cm^2) to the skin of male Wistar (Crl:WI)BR) rats (n=5). ^{14}C-Parsol SLX supplied by the sponsor was analysed by the testing laboratory to determine whether the radio-labelled test article was representative of Parsol SLX (identity confirmed). The vehicle for preparation and application of ^{14}C-Parsol SLX was PEG400. Prior to application of the test material the selected sites were clipped free of hair approximately 24 hours prior to dosing and application area/volume contained through the use of Plexiglass enclosure (semi-occlusive). All test animals were fitted (acclimatised previously) with Elizabethan collars to prevent removal of the test material during the 24 hours exposure period. The site of application was washed at 24 hours (exposure) after application of the test material. Plasma samples were collected for determination of ^{14}C-Parsol SLX at 2, 4, 8, 12, 24 and 48 hours after the start of dermal application; blood samples (approx. 0.4 mL) were taken from the jugular vein. Included in the analysis was an assessment of levels in urine and faeces collected over 3 time frames of 0-24, 24-48 and 48-72 hours after treatment. Recovery of administered ^{14}C-Parsol SLX was determined by collation of amounts found in urine, faeces, skin (application site) and carcass. The 5 animals used in this study were monitored twice daily for mortality, injury and use of food and water.

The composition of Parsol SLX test article was consistent with samples of ^{14}C-Parsol SLX. Results indicated that following the application of a single dose of ^{14}C-Parsol SLX to the skin of rats, plasma levels were below the limit of detection/quantification (2.5 ng equivalents/mL) at all analysis points. Urinary and faecal levels (recovery of radioactivity) from all animals had mean values of 0.2 (range 0.0-0.3%) and 0.4% (range 0.1-0.7%) of the administered dose, respectively. Measurement of radioactive residue in the carcass revealed a total of 1.5% (range 0.3-2.3%) of the administered was recovered. Analysis of the skin showed the greatest recoverable amount of radioactivity at an average of 48.9% (range 37.3-63.1%) of the administered dose. Total recovery of radioactivity appeared to be approximately 50%, which suggests that the remaining 50% was washed off the skin at 24 hours after application.

All animals were free of clinical signs of toxicity, and food and water consumption were apparently normal. It was concluded that dermal absorption was very low based on minimal amounts found in plasma, urine, faeces and carcass, while high levels of radioactivity were found in the skin. Any amounts of test material found in the faeces were likely to have come from cleaning/licking activities or from contaminate food. From the amount (0.2%) found in the urine it could be suggested that approximately 0.2% of the test material was absorbed following dermal application, despite plasma levels being below the level of detection.

> Excretion balance and pharmacokinetic study of radio-labelled Parsol SLX following oral administration to rats; s47F .; study number 968-003; s47F ; 2003; GLP/QA-yes.

The aim of this study was to determine the plasma pharmacokinetics of ^{14}C-Parsol SLX, following a single oral administration to male Wistar [Crl:WI)] rats. Radio-labelled Parsol SLX (suspended in canola oil) was administered by oral gavage as a single dose to two groups (5 animals/dose) at 2 or 1000 mg/kg. Blood was collected from all rats at pre-dose and 1, 2, 4, 8, 12 and 24 hours post-dose. In addition, to aid in the determine of total recovery urine and faeces were collected from 0-24, 24-48 and 48-72 hours after dosing to estimate the amount of label excreted and measurements of residues in the carcass were carried out. Cage rinses, wipes and washes were collected to aid in the detection of lost radioactivity. Doses

were based on levels used in a pilot kinetic study and the highest level used in the repeat-dose oral toxicity studies.

Preparation of the labelled material was affected by the suspension characteristics of the test material in canola oil, resulting in a higher concentration of radioactivity (6.54-6.72 μCi/kg low dose; 62.36-65.88 μCi/kg high dose) being present in the high dose of the test material. The dose of radioactivity was based on the amount of radioactivity considered necessary to detect radioactivity in the biological samples collected during the study. The test material was delivered in a volume of 3 mL/kg, with the bodyweight used in the calculation determined on the day of dosing. Animals were euthanized by CO_2 inhalation and the carcasses processed to determine residual radiolabel.

Animals were kept in appropriate conditions of food, water and day/night cycles prior to and during the study. Animals were monitored for morbidity, mortality, clinical signs of toxicity and food/water consumption throughout the duration of the study. Study animals were weighed pre-test and prior to dosing.

Plasma levels following the single oral dose of 2 mg/kg were found to be predominantly below the level of quantification (LOQ = 2.7 ng/mL) at all time points. Two out of five animals from this group showed marginal (right on 2.7 ng/mL) plasma levels only at 1 hour post-dose.

Plasma levels of radioactivity were measurable in all animals given the higher dose (1000 mg/kg), with a mean Cmax of 3174 ng/mL observed at 1 to 2 hours post-dose. Plasma levels in these animals dropped steadily to below the limit of quantification from between 8 to 12 hours post-dose in all animals.

Urinary recovery data following dosing with 2 mg/kg showed radioactivity from all animals was less (1.197% to 1.731%; mean 1.494%) than 2% of the administered dose, which is consistent with the finding of low plasma concentrations of radioactivity. The urinary recovery of radiolabel from animals treated with 1000 mg/kg was in the range of 0.652% to 0.855% (mean 0.707%). It was noted that a previous report from Roche (no. 1008016) identified urinary recovery of 0.3% to 0.4% of an orally administered dose. It was apparent from the data that urinary excretion did not vary appreciably between the two dose groups.

The faeces contained the greatest proportion of the excreted radioactivity. A mean concentration of 61.5% (42.5% to 76.3%) recovery was determined from animals receiving 2 mg/kg, while 75.0% (61.0% to 85.9%) was recovered from animals receiving 1000 mg/kg. It was suggested that the relatively low recovery of radiolabel in the faeces could be due to solubility problems encountered for the ^{14}C labelled test article.

Residual levels of radioactivity in the carcass at 3 days post-dosing were generally below the limit of detection. It was apparent from the kinetic profile of radio-labelled Parsol SLX that it is poorly absorbed following oral administration. It could be anticipated (using plasma concentration and urinary excretion data) that up to approximately 1.5% of the administered oral dose was absorbed. All animals were free of clinical signs of toxicity, and food and water consumption were apparently normal.

Analysis of the absorption of the test material in this study did not include plasma AUC estimates, which are accepted measures of levels of systemic exposure following administration by a chosen route/s (oral or dermal).

Meteor report (Computer generated prediction of the metabolic fate of chemicals; Lhasa Ltd – Predicting toxicity and metabolic fate; 2005).

Recently submitted in support of polysilicone-15 was a computer generated report from a program identified as Meteor, which it was stated, "help scientists who need information about the metabolic fate of chemical and want to be more efficient, more effective and make better decisions". A summary identified key features of Meteor, stating it predicts the metabolic fate of chemicals, displays results as a metabolic tree, covers phase I and phase II biotransformations, allows you to filter results and see only likely metabolites and links directly to MetaboLynx to speed up analysis of mass spectrometry data from metabolism studies. Included in this report is a diagram showing the predicted metabolic tree for polysilicone-15 (attachment 2) using Meteor (computer program).

Linked to the predicted formation of metabolites was an analysis (using Derek for Windows) of the potential toxicity of the metabolites suggested by Meteor. It was stated that Derek (for Windows) was a, "high throughput screen for genotoxicity/mutagenicity, it predicts whether a chemical will be a skin sensitiser, it predicts whether a chemical will be carcinogenic and it highlights potential toxicological hazards covering a wide range of endpoints from irritancy to hepatoxicity". Derek was described as an expert knowledge based system, which contains expert knowledge rules in toxicology and applies the rules to make predictions about the toxicity of chemicals, usually when no experimental data are available. The program applies structure-activity relationships [(Q)SARs] and other expert knowledge rules to derive a reasoned conclusion about the potential toxicity of the chemical. The program provides supporting evidence for its predictions. The supporting evidence includes comments, literature references and toxicity data.

Presentation of the parent compound and metabolite analysis was in the form of report sheets (attachment II), which included a section on "List of alerts found" for each compound. A total of 8 structures (related to polysilicone-15) were analysed, with no findings presented in the "list of alerts found" section for each of the structures. This would suggest that based on structure-activity relationships (Derek analysis), the possible metabolites formed from polysilicone-15 were identified as not likely to be hazardous. The predictability of structure-activity relationships regarding potential toxicity of compounds needs to be considered.

LOCAL TOLERANCE/PHOTOTOXICITY EFFECTS

Primary eye irritation study with GIV 84-5690 in rabbits; s47F s47F Switzerland; project no. 609344; s47F ; 1996; GLP/QA-yes.

The primary ocular irritation potential of GIV 84-5690 (batch no.248452) was examined following instillation of 0.1 mL into the conjunctival sac of one eye of each of 3 young adult NZ rabbits, while the untreated eye served as a control. The treated eyes were not rinsed after application. Assessment (scoring) of the degree of ocular irritation took place at 1, 24, 48 and 72 hours after application of the test material. Estimation of the irritation index involved using the scores from each reading point and calculating the respective mean values for each

type of ocular lesion. The primary irritation score (index) required all individual scores to be totalled and then averaged.

The results showed that the test material had a primary ocular irritation index/score of 0.67 following instillation directly into the conjunctival sac. This score was based on limited erythema and chemosis of the conjunctivae, while other structures (cornea, iris) were free of adverse effects. In all animals, slight erythema of the conjunctivae was generally seen up to 24 hours, but not at the 48 and 72 hours inspections. Detailed examination of the treated eyes found no evidence of staining of the cornea, sclera or conjunctivae. It was also noted that there was no evidence of corrosion. EEC derived criteria for classifying ocular irritation was applied to the irritation scores/index, with the test material found to be non-irritating to the rabbit eye.

> Primary skin irritation with GIV 84-5690 in rabbits (4 hours semi-occlusive application); s47F [redacted]. Switzerland; project no. 609333; s47F s47F [redacted] 1996; GLP/QA-yes.

The primary skin irritation potential of undiluted GIV 84-5690 (batch no.248452) was assessed in an assay using topical application of 0.5 mL of the test material to an area of 6 cm^2 on clipped intact dorsal skin of 3 NZ white rabbits (1 male, 2 females). The treated site was covered with a patch of surgical gauze and the gauze was covered with a semi-occlusive dressing. Exposure to the test material lasted for 4 hours after which time the dressing was removed and the test site was washed with lukewarm tap water. Irritation scores were determined at 1, 24, 48 and 72 hours after removal of the dressing. The individual irritation scores for each type of induced lesion for each of the assessment times were used to calculate a primary skin irritation index.

There were no apparent clinical signs of systemic toxicity in any animal over the course of the assay. Body weights of the study animals were within the normal range for this species and strain of rabbit. The primary irritation score/index was determined to be 0.11 (max score possible 8.0), which was related to scores of 0.11 from erythema and 0.0 from oedema. The irritation score (0.11) was due to 1 rabbit (female) showing very slight erythema at 24 hours after removal of the dressing. There was no evidence of corrosive damage to the skin at the application sites, while the treated skin showed no sign of staining as a result of exposure to the test material. The primary irritation score/index determined in this assay was consistent with a non-irritating rating according to the EEC Commission Directive 93/21/EEC.

> Determination of contact hypersensitivity in albino guinea pigs by the maximisation test to GIV 84-5690; s47F [redacted]. Switzerland; project no. 374995; s47F [redacted] 1994; GLP/QA-yes.

A preliminary concentration range-finding study was conducted to establish the highest non-irritating test article concentration to be used for the challenge in this assay. Based on the results a concentration of 100% was found to be appropriate, with no adverse effects (clinical signs of toxicity) evident during this assessment.

In the main section of this study, groups of Himalayan Spotted guinea pigs were subjected to an induction and challenge phase with GIV 84-5690. A treated group (n=20) and control (n=10) group were included in this assay. Data (generated Dec. 1993) from a non-concurrent positive control group was used to validate the sensitivity of the species and strain of animal

used. Induction involved administration of the test material intradermally at a concentration of 5% followed by undiluted test material applied topically (to the skin) under an occlusive dressing. An area of dorsal skin from the scapular region (approx. 50 cm^2) was clipped free of fur. During the induction Freund's Complete Adjuvant (FCA) was employed as a vehicle for the test material to sensitise the induction system. Three pairs of intradermal injections (0.1 mL/site) were carried out at the border of the prepared region on test day 1. In the test group animals, these 3 sites coincided with application of FCA (site 1), the test material and FCA (site 2) and the test material alone (site 3). The control group received FCA plus the vehicle ethanol or just FCA (in saline). Parameters assessed during the study included formation/occurrence of erythema and/or oedema, which was graded according to the Draize classification. On test day 7, the application sites were clipped free of fur and pretreated with 10% sodium lauryl sulfate (SLS). On test day 8, a patch of filter paper (8 cm^2) was saturated in the test material and placed over the three injection sites located on the dorsal region of the test animals.

At challenge on test day 22, topically (dermal) applied test material at 5%, 10%, 30% and undiluted was used to try and elicit a response. The application sites were re-clipped prior to application of the test material. Patches (4) of filter paper (4 cm^2) were saturated in the test material and placed over the injection sites located on the dorsal region of the test animals. Application and fixing of the patches involved the same method described for fixing of the induction patches. These challenge patches were left in place for 24 hours. Scoring of skin reactions to the challenge treatments took place 24 and 48 hours after removal of the patches and followed the Draize system.

In general, the condition of the animals in this study was very good with no deaths and no evidence of systemic toxicity. All animals had body weights within the normal range for the species and strain. Necropsies were only scheduled for animals that died during the study and since there were none there was no data generated examining gross pathology.

Skin reactions to the vehicle (ethanol) and GIV 84-5690 assessed at 24 and 48 hours after topical application were negative with no evidence of either erythema or oedema in any animal. The control group did not show any skin reactions/responses to challenge at either 24 or 48 hours after removal of the dressing. Similarly, the test group showed no evidence of any skin reactions at either the 24 or 48 hours challenge. The non-current positive control group (4-aminobenzoic acid ethyl ester 25% in mineral oil) had generated a significant skin reaction (limited to erythema 1-2 intensity, indicating mild sensitiser) at challenge in this species and strain of animal at both analysis (24 & 48 hours) in approximately 50% of animals.

Overall, GIV 84-5690 was not a skin sensitiser in a maximisation assay at a challenge concentration of 100% applied topically.

> Determination of photo-toxicity with GIV 84-5690 in albino guinea pigs; s47F s47F. Switzerland; project no. 609355, s47F; December 1995; GLP/QA-yes.

A single dose of GIV 84-5690 (25%, 50%, 75% and 100%) was applied to guinea pig skin prior to UV irradiation at photo-toxicity test sites and non-irradiated (control) sites. The left flank of the animal was irradiated with 20 J/cm^2 of UVA radiation, while the right flank was the non-irradiated control site. In preparation for this test, the animals were clipped free of fur at the application sites. A volume of 0.025 mL/2 cm^2 of the test articles were applied at

concentrations of 25%, 50%, 75% and 100%. Approximately 2 hours after application of the test article the left flank was exposed to a non-erythemogenic dose of UVA radiation. The right flank received the test article but not UVA irradiation. Reactions at these sites were assessed for potential photo-induced toxicity. During the study animal survival was monitored, along with body weights, clinical signs of toxicity and skin reactions. An autopsy was not performed on any of the study animals. Data from a non-current positive control was included in the study description (conducted Oct. 1995). The positive control was 8-methoxypsoralene (0.03-1%), which had been used to examine the appropriate responsiveness of the species/strain of animal used in this study. Reactions to the positive control were seen at every concentration and analysis point indicating the species/strain of animal used gave appropriate responses.

There were no deaths or visible clinical sign of toxicity during the course of the study. Body weights were similar in test animals and controls and there was no evidence of skin reactions at either the test or control sites. Overall, there was no evidence of photo-toxicity as a result of exposure to GIV 84-5690 in the presence or absence of UVA radiation.

> Determination of photo-allergenicity in albino guinea pigs to GIV 84-5690; s47F s47F. Switzerland; project no. 383883, s47F; January 1995; GLP/QA-yes.

This study assessed the photo-allergic potential of GIV 84-5690 in a maximisation assay using Himalayan spotted SPF guinea pigs (10 control & 20 test group animals). A further 4 animals were employed in a pre-test assessment of appropriate concentrations of test material to use in the main study.

In the induction phase of the main study, 0.1 mL GIV 84-5690 (100%) was applied epicutaneously to an area of skin (previously shaved) approximately 8 cm^2 in size, which had previously been pre-conditioned using 4 intradermal injections (0.1 mL) of Freund's Complete Adjuvant (FCA). The test sites were then irradiated (using Philips Actinic TLD lamps) with 10 J/cm2 UVA and 1.8 J/cm2 UVB total exposure. This procedure was repeated 4 times within the 2 weeks induction phase on days 3, 5, 8 and 10, while control animals were treated with FCA only (no UVA or UVB radiation). Challenge was initiated 3 weeks after commencement of the induction procedure and consisted of epicutaneous application of GIV 84-5690 at concentrations of 25%, 50%, 75% and 100% to both flanks of the animals. Treated sites of the left flank were then exposed to 10 J/cm2 UVA irradiation, while the right flank was not irradiated and acted as a control for sensitisation reactions (minus UV exposure). All sites were assessed for skin reactions (eg. erythema, oedema) at 24, 48 and 72 hours after the challenge.

It was noted that a positive control (0.003-1.0% 3,3',4'5-tetrachlorosalicylanilide) is tested once a year to establish the sensitivity of the species/strain of animal used in sensitisation assays. The most recent assessment using the positive control was in June 1994, which showed a positive photo-sensitisation response in up to 90% of test animals at the highest concentration.

Over the course of the study the condition of the study animals was monitored, including mortality, body weight, as well as clinical signs/symptoms of toxicity and skin reactions. Necropsy was only performed on animals that died prematurely.

A test animal that died on day 23 was examined for a cause of death and was found to have a dark red discolouration of the lungs (not treatment-related). There were no clinical signs of toxicity displayed during the course of the study and body weights were found to decrease across both groups (test and control) between acclimatisation and treatment (deemed incidental). Skin reactions were observed after injection of FCA, but application of the test material had no apparent adverse effect of the skin (no reactions observed).

A table of results for irradiated test and control groups compared with non-irradiated test and control groups for all analysis points and concentrations was presented, which indicated there were no positive responses (test and control animals) observed at any analysis point during the course of the study. There was no evidence of a sensitisation or photo-sensitisation reaction in this study under the conditions described above.

ACUTE TOXICITY

Acute oral toxicity study (fixed dose method) in rats with Ro84-5690/001; s47F s47F s47F Switzerland; study protocol no. 170 G 94; s47F ; 1994; GLP/QA-yes.

A single group of 5/sex (total 10) Hanlbm:WIST(SPF) rats (approx. 6 weeks old) was used in this acute oral toxicity study, where Ro84-5690/001 (batch no. 231039) was administered by gavage at 2000 mg/kg. The test material was suspended in a standard suspension vehicle (SSV) consisting of sodium carboxy methyl cellulose, Tween 80, benzylalcohol, sodium chloride and water. The animals were fasted prior to administration of the test material. Animals were observed for a period of 14 days following administration of the test material. Parameters checked included clinical signs of toxicity, body weight, mortality and an examination for gross lesions at necropsy.

No deaths occurred and no clinical signs of toxicity were observed during the course of the study. Body weight development was within the normal range for all animals and there were no abnormal findings at necropsy. The oral LD50 value was determined to be >2000 mg/kg.

Acute dermal toxicity study (limit test) in rats with Ro84-5690/001; s47F s47F . Switzerland; study protocol no. 179 G 95; s47F 1995; GLP/QA-yes.

A single group of 5/sex (total 10) Hanlbm:WIST(SPF) rats (approx. 6 weeks old) was used in this acute dermal toxicity study, where liquid Ro84-5690/001 (batch no. 248452) was administered topically to the skin at 2000 mg/kg over an area (10% of surface) of 16 cm^2. Exposure was for 24 hours at which time the test material was cleansed from the skin using a solution of ethanol (70%) in water. Animals were observed for a period of 14 days following administration of the test material. Parameters checked included clinical signs of toxicity, body weight, mortality and an examination for gross lesions at necropsy.

No deaths occurred and no clinical signs of toxicity were observed during the course of the study. The site of application of the test material remained free from adverse effects during the observation period. Body weight development was within the normal range for all animals and there were no abnormal findings at necropsy. The dermal LD50 value was determined to be >2000 mg/kg.

REPEAT-DOSE/SUBCHRONIC TOXICITY

Ro 84-5690/001: thirteen week oral (gavage) toxicity study in rats; s47F s47F . Switzerland; study protocol no. 152 G 95; s47F 1996; GLP/QA-yes.

In this study, groups of Wistar rats (20/sex in control and high dose groups, 10/sex in low and mid-dose groups) were administered Ro 84-5690/001 orally by gavage at doses levels of 0, 60, 220 or 1000 mg/kg/day for a period of 90 days. The vehicle control group received rapeseed oil without the test material. Parameters measured/assessed during the course of the study included clinical signs of toxicity, survival, body weights, feed consumption, haematology, clinical chemistry, urinalysis, organ weights, and micro- and macroscopic examination of tissues at scheduled sacrifice. At the completion of the 90 days treatment period a number (9 males and 10 females) of animals from the control and high dose groups were followed for a further 4 week drug free recovery period. These recovery animals were subjected to the same observation/assessment procedures as those conducted during the treatment period.

Almost all animals survived to scheduled sacrifice with no overt evidence of systemic toxicity based on an absence of clinical signs of toxicity. A total of 2 deaths occurred, with 1 control male and 1 HD male found dead on days 39 and 84, respectively. Both these animals were free of overt signs of toxicity prior to their death and there were no obvious pathological changes seen at necropsy. It was concluded that the death of the HD male was not due to treatment with the test material, although the cause of death not clearly established. The control male had obstructive uropathy, which may have been the reason for his death.

There were no treatment-related adverse effects on body weights, feed consumption, urinalysis parameters, ophthalmological function, haematological parameters, macroscopic or microscopic pathology. All haematological parameters remained within the normal physiological range established for this species and strain. Minor transient changes (not dose-related) seen in some parameters registered as statistically significant, but were of no biological or toxicological significance. Haematological data from the recovery groups were similar to the data/values measured at the end of treatment (days 86-87).

Organ weight analysis revealed minor differences in relative (to body weight) liver weight (increased in HD males only), as well as some variations in clinical chemistry findings across the groups. The increase in relative liver weight was 16% in the HD male group, while the LD and MD groups were slightly above the control value. Analysis of the liver weight after the drug-free recovery period showed a value marginally (6%) above the control value, indicating a change toward control levels. Histopathological analysis did not reveal any adverse changes in the liver at any dose level compared to the control group. The change in liver weight appears to be of no toxicological concern since it was not accompanied by structural changes. It was proposed that the increase in liver weight possibly represented an increased demand for liver function to eliminate the test substance. The possibility that this response was due to the vehicle (rape-sed oil) was unlikely, since the relative liver weights in the control group were within normal range and formed the baseline for assessed changes (HD increased relative to control).

Clinical chemistry changes included effects on serum concentrations of total bilirubin, aspartate aminotransferase (AAT) and alkaline phosphatase (ALP). It was noted that the

biotransformation of unconjugated bilirubin (which is albumin-bound in plasma) takes place in the liver. The accelerated elimination (associated with decreased plasma bilirubin) of serum bilirubin showed that the hepatic bilirubin metabolism was increased. Also, the metabolism of xenobiotics is often associated with bilirubin metabolism, the decreased serum bilirubin concentration was considered to be a sign of metabolic adaptation to exposure to the test material. Bilirubin was decreased in male groups by 6% to 30% and in female groups by 4% to 18%. These changes only reached significance in the MD (only in females) and HD groups, but in a dose-related manner, while no significant differences were observed in bilirubin concentration after the drug-free period (recovery groups, control and HD) for either sex. Reductions in activity/concentration of liver enzymes are meaningless in the context of biological activity indicating that the changes seen in this study would not represent a manifestation of toxicity. Levels of these marginally (<30% in all cases) altered parameters were similar in HD animals and controls at the analysis of the drug-free period.

A slight reduction in albumin/globulin ratio occurred across treatment groups and was related to a decrease in serum albumin levels. The reduction was not dose-related and was no longer evident after the drug-free recovery period.

Overall, the changes in these parameters were considered by the investigators to reflect hepatic adaptation to the test substance and it was suggested the changes were of no toxicological significance.

Data presented showing analysis of tissues and organs were unremarkable, with no evidence of any treatment-related changes in any group compared with the controls. The only finding of note was the incidence of obstructive uropathy in a control male, which was likely to be linked to this animal's death.

It was concluded that the NOEL for this study was the highest dose used which was 1000 mg/kg/day.

REPRODUCTIVE TOXICITY

> Transactivation assay in stably transfected MCF-7 cells in order to determine the oestrogenic potency of Ro 81-6462/000 and Ro 84-5690/001; s47F [redacted] s47F [redacted] study no. 8740/202; s47F [redacted]; March 2002; statement indicating good scientific practice included, no GLP/QA.

In this study, two UV filters were examined for potential functional oestrogen mediated transactivation, which could be linked to oestrogenic activity. The system used in this study was transfected MCF-7 cells, constitutively expressing both oestrogen receptor isoforms ER and ER stably transfected with a reporter gene under control of the oestrogen regulatory element (gene promoter). In this model, oestradiol induces a marker (luciferase) in a dose-dependent manner at sub-nanomolar concentrations, with maximum levels of this marker reached by 24 hours into incubation of cells and oestradiol.

In this assay, MCF-7 cells were incubated over 24 hours with Ro 84-5690 and another UV filter (separate assay), which were dissolved CDFCS-medium containing 0.1% DMSO at concentrations of 1nM to 10µM. Controls included were oestradiol (positive, 0.01pM to 100nM) and CDFCS-medium containing 0.1% DMSO (negative), while a further check of system viability (oestrogenic activity) included the use of an oestrogen antagonist (ICI

182780, 1µM). A total of 5 concentrations (1, 10, 100, 1000 & 10000nM) of the UV filters were tested, with the assays performed 3 times in independent experiments.

Measurement of the marker (luciferase) following incubation with the positive control substance found significant dose-dependent increases in oestrogenic activity, which was antagonised by the oestrogenic antagonist. There was no evidence of oestrogenic (induced luciferase) activity following incubation with either of the UV filters assessed in this study. It was concluded that Ro 84-5690/001 did not possess the capacity to induce oestrogenic activity in transfected MCF-7 cells *in vitro*.

Ro 84-5690/001 segment II: oral study for the effects on embryo-foetal development in the rat; s47F s47F Switzerland; study protocol no. 456 R 99; s47F ; December 1999; GLP/QA-yes.

Ro 84-5690/001 was assessed for its potential teratogenic activity in groups (25/dose) of mated female Wistar (RORO) rats at doses levels of 0 (vehicle only), 100, 300 or 1000 mg/kg/day. Ro 84-5690/001 was administered by gavage (orally in rape seed oil) from gestation day 6 through to gestation day 20. All study animals were sacrificed on day 21 of gestation and their uterine contents removed for investigation (for external, visceral and skeletal abnormalities). Parameters followed during the course of the study included maternal toxicity (survival, body weight, food consumption, clinical signs of toxicity and necropsy), reproductive and foetal data, and detailed external and internal foetal pathology.

In general, there was no evidence of maternal toxicity during the study. All values and observations for clinical signs of toxicity, food consumption, body weights, survival and findings at necropsy were similar across all groups including the controls. All reproductive parameters were unaffected by Ro 84-5690/001, while there were no significant treatment-related effects on external, visceral or skeletal abnormalities, variations or delayed development of growth in foetuses.

As mentioned below, there were fewer pregnant females at the MD and HD than in the LD group and controls. Monitoring of clinical signs revealed no changes from days 0-5 of gestation, while individual dams from all groups showed hair loss or hair thinning from days 6-17 (dosing period) and days 18-21 of gestation. Body weights of pregnant females increased in a similar manner across all groups (median values 106, 106, 110 and 108 g for the control, LD, MD and HD groups, respectively). Food consumption was consistently 20 g/animal/day over the course of the study with one exception, which was a consumption of 21 g/animal/day during the gestation period of 0-7 days for MD animals. At necropsy, 1 LD dam had a cyst in the kidneys and worms were found in the intestine of 2 dams (1 HD and 1 MD dam).

Reproductive data showed that 25, 25, 22 and 23 of the mated females from the control, LD, MD and HD groups, respectively, were found to be pregnant; the following data refers to all pregnant females. Although there were slightly fewer pregnant females in the MD and HD groups this difference had nothing to do with treatment as mating occurred well before administration of Ro 84-5690/001 started. The median number (per pregnant female) of corpora lutea (13-14), implantations (12-14), live foetuses (11-13), dead foetuses (0), resorptions (0-1) and weight of foetuses (4.9-5.0 g) were unaffected by treatment. Analysis of the sex ratio showed a greater number (not significant) of males than females in the control group, while all the treatment groups had more females than males.

Total foetal abnormalities were assessed and showed an incidence amongst foetuses of 2.9, 1.8, 0.7 and 3.1% for the controls, LD, MD and HD groups, respectively. The incidence of these changes was spread across 2-6 litters. Total foetal variations were assessed and showed an incidence amongst foetuses of 17.9, 19.5, 18.8 and 18.2% for the controls, LD, MD and HD groups, respectively. The incidence of these changes was spread across 17-20 litters. Total foetal developmental retardations were assessed and showed an incidence amongst foetuses of 5.8, 7.0, 5.9 and 7.4% for the controls, LD, MD and HD groups, respectively. The incidence of these changes was spread across 10-14 litters. External observations of changes showed incidences of lordosis, short trunk, limb hyperflexion, peromelia, limb shortened, hindlimbs originate asymmetrically and tail shortened or rudimentary. The only change that occurred in more than 1 foetus from a group was the incidence of tail shortened/rudimentary, with 5 foetuses from the control group presenting with this change. Visceral changes included brachiocephalic trunk change, abnormal origin of artery, renal papilla reduced, convoluted ureter and hydro-ureter; the incidence of these change was low across all groups. Skeletal changes to the skull, cervical vertebra, thoracic vertebra, lumbar vertebra, sacral vertebra, caudal vertebra, sternum, ribs, forelimbs, phalanges fore and hind, hindlimbs, metatarsals and pelvis were noted. The vast majority of these changes occurred only in 1 individual from any group, while higher incidences of changes occurred at a similar rate across all groups.

Overall, it was apparent that Ro 84-5690/001 at doses up to 1000 mg/kg/day administered orally during gestation did not cause maternal toxicity and had no adverse effects on reproductive parameters or the development of the foetuses.

GENOTOXICITY

Mutagenicity evaluation of Ro 84-5690/001 in the Ames test; s47F , s47F s47F . Switzerland; study protocol no. 133 M 95; s47F ; Sept. 1995; GLP/QA-yes.

The mutagenic potential of Ro 84-5690/001 was examined in the Ames test using standard plate incorporation and pre-incubation (modified) methods. In the assays 6 strains of *Salmonella typhimurium* (TA1535, TA1537, TA97, TA98, TA100 and TA102) and one strain of *Escherichia coli* (WP2 uvrA) were used to assess possible point mutation effects. The assay incorporated metabolic activation prepared from treated rat livers and positive and vehicle control groups were included in the study design. Positive controls included sodium azide, 2-nitrofluorane, ICR 191 and 2-aminoanthracene, with results for these agents generating significantly greater mutant frequencies across all strains. It was indicated that the concentrations of the test material would be based on the generally recommended highest test concentration for non-toxic substances, which is up to 5000 µg/plate in the presence and absence of metabolic activation. No cytotoxicity was observed at the highest concentration of 5000 µg/plate.

There was no evidence of an increase in the number of revertant colonies for any of the cultured strains in the presence or absence of metabolic activation. Analysis for differences between treated and control revertant frequencies did not produce a single significant fluctuation (or concentration-related trend) for any strain in the presence or absence of metabolic activation. The mutant frequencies were in the range seen in the historical controls and published data in the literature. The positive control induced a significant increase in the

frequency of mutant colonies verifying the sensitivity of the strains used. Ro 8405690/001 was shown not to possess mutagenic activity in this assay.

Chromosome analysis in cultured V79 Chinese hamster cells treated with Ro 84-5690/001 in the presence and absence of a metabolic activation system; s47F - s47F . Switzerland; study protocol no. 136 M 95; s47F ; January 1996; GLP/QA-yes.

Ro 84-5690/001 was evaluated for potential clastogenic effects on cultured V79 Chinese hamster lung cells in the presence and absence of metabolic activation (rat liver S9). In pre-testing, concentrations (50-5000µg/mL) of Ro 84-5690/001 were not cytotoxic as determined by a relatively unaffected mitotic index and number of cells. It was indicated that the concentrations of the test material would be based on the generally recommended highest test concentration for non-toxic substances, which is up to 5000 µg/mL in the presence and absence of metabolic activation. Positive (bleomycin 1-2 µg/mL & cyclophosphamide 0.2-0.5 µg/mL) and vehicle controls were included in this assay.

Incubation of the test material with the cultured cells did not result in an increase in structural (or frequency) chromosome aberrations that could be considered of statistical or biological relevance in either the presence or absence of metabolic activation. Results following exposure to the positive control indicated a significant increase in the frequency of structural chromosomal aberrations thus validating the sensitivity of the assay system. The negative control values were within the generally accepted historical range for this cell type. It was concluded that Ro 84-5690/001 was not clastogen *in vitro* in the assay described above.

Ro 84-5690/000: mouse lymphoma cell mutation test (ML/TK); s47F UK; research report no. B-167'778, (study no. 948 M 97); s47F ; January 1998; GLP/QA-yes.

Ro 84-5690/000 was assessed for its potential to induce mutations at the *tk* (thymidine kinase) locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a ML/TK test. Included in this study were a cytotoxicity range-finder evaluation followed by duplicate assays, each conducted in the presence and absence of metabolic activation. The vehicle for the test material was ethanol and a positive control (4-nitroquinoline 1-oxide; benzo[a]pyrene) was included to validate the test system. The test material was tested at concentrations much higher than its solubility limit in the culture medium (up to 1500 µg/mL), with this concentration used as the high concentration in the main assays. Maximum concentration (1500 µg/mL) for the main assays was selected based on a finding of no functionally significant cell loses (survival 60.8-91.1% ±S9) at this level in the range-finding assay.

Concentrations used in the main assays ranged from 11.7-1500 µg/mL in the presence and absence of metabolic activation and time for incubation/exposure in culture was 3 hours. Cell survival in these assays did not drop below 72% at any concentration.

In the negative control, mutant frequencies fell within normal ranges, and clear increases in mutation frequency were induced by the positive controls. Data for the vehicle and negative cultures were similar. Results showed no increases in mutant frequency compared to the negative/vehicle control values in the duplicate assays in presence or absence of metabolic activation. Responses to the positive control agents verified the sensitivity of the test system.

The test material was not mutagenic in mouse lymphoma cells using a ML/TK test at concentrations up to1500 µg/mL.

Photo-mutagenicity evaluation of Ro 84-5690/001 (Parsol SLX) in the yeast *Saccharomyces cerevisiae* D7 exposed concomitantly to simulated solar radiation; s47F s47F .s47F . Switzerland; study protocol no. 132 M 95; s47F ; Sept. 1995; GLP/QA-yes.

In this study yeast *Saccharomyces cerevisiae* D7 (diploid yeast strain) cultures were exposed to Ro 84-5690/001 and UV radiation (Suntest CPS light source + filter) test for potential photo-mutagenicity activity. *Saccharomyces cerevisiae D7* (cultured in YEP medium) was used as it provides a system that has several genetic end-points that can be scored which reflect potentially harmful interaction (chemical/UV) within the cells. Concentrations of Ro 84-5690/001 tested ranged from 10 to 1000 µg/mL and UVB/A radiation (doses up to 150mJ/cm^2) were used to investigate possible induction of photo-mutagenicity. This UV dose was selected as a dose inducing an approximate increase in conversion frequency of 3 to 4 fold over background levels. Positive (chlorpromazine) and vehicle (ethanol) controls were included in this assay. Criteria for acceptance of a substance as a photo-mutagen was; if in the presence the mutant frequency surpasses the mutant frequency observed in its absence at the same irradiation time.

The data showed that there were no photo-mutagenic effects of Ro 84-5690/001, with the frequencies of convertants similar in controls and test cultures before and after irradiation. It was suggested that the test material actually offered a protective effect, with fewer convertants detected in the presence of Ro 84-5690/001 after irradiation. A clear response to the positive control verified the sensitivity of the test system.

The data indicated that Ro 84-5690/001 was not photo-mutagenic in *Saccharomyces cerevisiae D7* cells under the conditions described above.

Photo-clastogenicity evaluation of Ro 84-5690/001 (Parsol SLX) in cultured V79 Chinese hamster cells exposed concomitantly to simulated solar radiation; s47F s47F .s47F . Switzerland; study protocol no. 136 M 95; s47F ; January 1996; GLP/QA-yes.

Ro 84-5690/001 (up to 5000 µg/mL) was assessed for potential photo-clastogenic activity in cultured V79 Chinese hamster lung cells that were irradiated (light source Xenon burner) with UVA/B doses of 100/2.0 to 500/6.3mJ/cm^2 *in vitro*. Initial cytotoxicity testing was conducted, which showed that concentrations of the test material up to 5000 µg/mL were not toxic based on mitotic index data. Ro 84-5690/001 was added to the cultured cells as either a suspension (up to 2000 µg/mL) or a mixture with oily drops (up to 5000 µg/mL). Included in the study were positive (chlorpromazine), vehicle (ethanol) and negative control groups. Prior to evaluation of the cultures the spindle poison colcemid (0.2 µg/mL 2.5 hours before harvesting) was added to each slide culture to arrest metaphases and allow chromosome preparation and staining. Scoring for structural aberrations was conducted on the positive control initially to establish that the system was functioning appropriately. Approximately 100 metaphases from selected slides were analysed for numerical aberrations.

Protocols for the treatment regimens were, 1. UV clastogenicity in the absence of Ro 84-5690/001, 2. clastogenic activity of Ro 84-5690/001 in the absence of UV radiation, 3. clastogenic activity of Ro 84-5690/001 in the presence of UV radiation, and 4. clastogenic activity of positive control. The sensitivity of the test system was confirmed by the response induced by the positive control with and without UV radiation. In the absence of UV radiation the scores for aberrations/cell were similar across all concentrations of Ro 84-5690/001, and the positive and vehicle control groups. In the presence of UV radiation, all concentration of Ro 84-5690/001 and the vehicle control group had similar levels of aberrations, while the positive control induced significantly more aberrations.

CARCINOGENICITY

No data were submitted addressing the issue of potential carcinogenicity. A rationale for absence of carcinogenicity data was submitted, which is present and discussed in the summary/assessment section of this report.

CLINICAL DATA/INFORMATION

Human repeated-insult patch test for contact sensitisation; s47F s47F UK; study no. GRS/01/V; s47F ; October 1996; GCP/QA-yes.

The stated aim of this study was to investigate the potential skin irritation and contact sensitisation activity of Ro 84-5690 (UV filter) in human volunteers. Ro 84-5690 was prepared for use as a 10% w/w solution in mineral oil on a daily basis. A volume of 0.4 mL 10% test solution was applied to cotton squares, which were applied to the upper outer arm and covered with an occlusive dressing/tape. The panel of subjects was selected based on a series of inclusion and exclusion criteria. A panel of 103 subjects (83 female and 20 male) was broken down into subgroups where 86 achieved programmed induction (8 induction treatments), 13 subjects had 6 or 7 induction treatments and 4 apparently did not complete the induction process. Induction involved 7 occlusive 24 hour and 1 occlusive 23 hour exposures to the upper outer arm over a period of 3 weeks. Once the induction process was completed there was a 14 days non-exposure period, which was followed by challenge application of the test material (10%). Challenge involved duplicate 24 hour exposures with the test material applied to both the original site and a naive site (on different arms). It was noted that a total of 92 subjects completed the challenge process. Assessment of reactions took place at 24, 48, 72 or 96 hours after each induction dose and approximately 48 and 96 hours after the challenge application. Skin assessment was based on a scale of 0, 1, 2 and 3 that relate to no visible relevant reaction, slight but distinct erythema, moderate erythema and strong erythema, respectively. Other skin reactions were identified by a code for oedema, papules, etc.

Results indicated that of the 103 subjects to start the study a total of 92 completed the study in a satisfactory manner; subjects not finishing the study cited varying reasons including work commitments to health problems. Consideration of the withdrawals noted that none of the reasons related to a problem with the test material. A breakdown of the subjects who completed the study showed 81 subjects had 8 induction treatments and 11 subjects had 6 or 7 induction treatments.

The table of results for skin reactions showed a total absence of erythema or any other type of skin reaction throughout the induction period indicating that the test material did not cause

skin irritation. A similar result was observed during the assessment of reaction to challenge, with no evidence of erythema or any other skin reaction at either challenge time or at treated or naive sites. Overall, it was apparent that the test material (10% solution) did not cause skin irritation or skin sensitisation under the conditions described above.

Photosensitisation potential in humans; s47F ; s47F s47F ; study no. 591851 (report no. 14470); December 1996; GLP/QA-yes.

The photosensitisation potential of Parsol SLX (10%) was assessed in 30 subjects (23 females and 7 males; skin types 1-3) according to the method developed by Kaidbey and Kligman (Cont Derm; 1978; 4; 277-282). The test material was submitted in solution for testing with a certificate of analysis. Vehicles (cyclomethicone and dimethicone copolyol) to be used to prepare the test material for dosing were included in the substances submitted to the testing laboratory. The subjects were identified as healthy human volunteers that met a criteria enforced (skin condition) at a pre-test examination. The UV source used in this study was a 1000 W Xenon arc lamp solar simulator, which was filtered to produce UV radiation in the UVA/UVB range. The intensity of the output of the lamp was monitored by a UVX radiometer and in conjunction with a UVX-31 sensor to measure emitted UV radiation.

In pre-testing, a minimal erythemal dose (MED) was determined for the subjects and the measured value formed the basis for exposure times required during induction. Induction involved application of the test material to sites (each 3 x 3 cm) on the upper mid-back of each volunteer. These sites were marked to ensure the repeat dosing during induction was applied to the same site. A second site (test material control) for each subject was prepared as previously mentioned, but this site did not undergo UV irradiation. Application of the test material was kept constant at 10mg/cm^2, which equated to a dose of 90 mg at each test (+UV) and control (-UV) site. An occlusive dressing was placed over the sites, with application of the test material at the 2 sites on days 1, 4, 8, 11, 15 and 18. Irradiation of these sites (+UV) took place 24 hours after application of the test material following removal of the occlusive dressing/patch. The dose of UV radiation varied from a MED of 1 on the first 2 days, a MED of 2 on the middle 2 days and a MED of 3 on the final 2 days. Skin reactions to the test material and UV radiation were assessed on days 4, 8, 11, 15 and 18.

At challenge, which took place 10 days after the last induction treatment, the test material was applied to the test and control sites as described above and both sites were covered with an occlusive dressing. In addition, a further untreated site served as an irradiation control. After a period of 24 hours the dressings were removed and the test and irradiation control sites were irradiated with 10 J/cm^2 of UVA. Each of the test and control sites was examined at 24, 48 and 72 hours for signs of skin reactions such as erythema/oedema.

Recorded observations showed that there were no adverse reactions to Parsol SLX at any time during the study. There were no reactions observed at the test material control sites in any subject during either the induction or challenge phases. During induction, all subjects showed a reaction (erythema) at the test material plus UV test site, with all subjects showing a reaction (of equal or greater intensity) at the control UV site. A total of 9 subjects displayed a slight reaction at the test material plus UVA test site at challenge, but these same subjects displayed a similar reaction at the UVA control site. Overall, it was apparent that the test material did not induce a photosensitisation reaction and that any reactions observed were due to the effects of UV radiation.

OTC MEDICINES SECTION

Supplementary data for polysilicone-15 UV filter.

The new ingredient in question is polysilicone-15 (Parsol SLX), which is a new UVB filter for use in primary and secondary sunscreens in Australia. At the October 6th 2005 Meeting of MEC the committee indicated that there were issues that needed to be addressed prior to approval of this new UVB filter. The sponsor was requested to provide the following additional information in support of their application:- an *in vivo* genotoxicity assay, further pharmacokinetic analysis of the parent molecule and its side chains and a three month dermal toxicity study. s47 [redacted] have provided additional information in response to requests by MEC.

Assessment

The sponsor was requested (MEC minutes, attachment 1) to provide the following additional information in support of their application:- an *in vivo* genotoxicity assay, further pharmacokinetic analysis of the parent molecule and its side chains and a three month dermal toxicity study.

An *in vivo* mouse micronucleus study was submitted, which met all current standards of GLP and quality assurance. Parsol SLX at oral doses up to 2000 mg/kg did not induce an increase in micronuclei above control levels indicating it was not genotoxic (clastogenic) in this assay. This *in vivo* result added to the negative results already seen in a series of *in vitro* assays assessing different end points.

Computer generated assessment (*in silico* by METEOR) of the metabolism of polysilicone-15 was extended to examine the benzylidene malonate groups attached to the polysiloxane backbone. s47 [redacted]

s47 [redacted]

Analysis of the formation of metabolites following an oral pharmacokinetic study in rats was limited due to low recovery. The formation of metabolites as analysis by METEOR showed no alerts for probable metabolites (likely to occur), while plausible (less likely to occur) metabolite alerts were discounted based on *in vivo* and *in vitro* data. The likelihood that hazardous metabolites are formed and are available to the systemic circulation appears negligible to minimal.

A three months dermal toxicity study was not submitted, but a justification for not submitting this study revolved around a daily sub-erythemal exposure study in humans, updated history of use in marketed products and an expert report on properties of polysilicone-15. The sub-erythemal exposure study in humans with susceptible skin (type I/II) was limited by its short duration (11 days) and low number of subjects (n=6), but it did show that the presence of polysilicone-15 (and butyl methoxydibenzoylmethane) significantly reduced local markers of skin damage. Analysis revealed indices of DNA damage, p53 expression and Langerhan cell function were altered at irradiated vehicle sites, while sunscreen (containing 6% polysilicone-15) protected and non-irradiated sites had similar levels of markers. Assessment for the presence of sunburn cells, apoptosis and epidermal proliferation was unremarkable. It was suggested that the use of a sunscreen (includes polysilicone-15) had a positive effect on cell viability and no obvious detrimental effect. However, the primary result from this study is that a sunscreen can protect against sub-erythemal UV damage.

Further supporting information related to the usage of polysilicone-15 in marketed products. At the time of the initial submission of the data package for polysilicone-15 there had been limited public exposure to polysilicone-15 in products. It has now been used in Europe since 2001. Between 2001 and 2005 (inclusive) s47 [redacted] over this period of time there has been no adverse effects reported to the sponsor/manufacturer.

The expert report (data for all cited studies provided) identified issues with currently approved (all grandfathered) organic UVB filters and suggested that these substances were not without unwanted aspects/behaviour and new UVB filters were needed. He noted that the most commonly used organic UVB active (octyl methoxycinnamate) in sunscreen products had relatively high percutaneous absorption (2%) and shows photo-instability; polysilicone-15 had a percutaneous absorption of approximately 0.2% and was photo-stable. Also another UVB active showed greater photo-instability in the presence of the most commonly used organic UVA active resulting in the inclusion of other UV filters to compensate for loss of protective activity. The report highlighted the ability of polyislicone-15 to provide photo-stability to other UV actives following exposure to large doses of UV radiation. A second study was described that indicated a synergistic effect with the use of polysilicone-15 improving the SPF, which was linked to photo-stabilisation.

The expert also pointed that the usefulness of a sunscreen is associated with the compliance or proper use (correct application amount) of the sunscreen. Cosmetic appeal has an impact on the use of sunscreens. A study showed that the addition of polysilicone-15 to an inorganic active (zinc oxide or titanium dioxide), compared to inorganic active alone, resulted in 29% more sunscreen being applied to the skin offering potentially better protection. In addition, according to the expert higher SPF values can be achieved using a combination of inorganic and organic UV actives, better cosmetic acceptability (better compliance) occurs, cost effectiveness improves and a combination facilitates compliance with the broad spectrum standard (AS2604). The use of an organic UV active with low percutaneous absorption and

photo-stability, such as polysilicone-15, has merits according to the expert. He pointed out that polysilicone-15 (liquid) has physical properties consistent with better product formulation; the use of polysilicone-15 should not be associated with altered product performance due to crystallisation or layer separation on storage.

NICNAS have approved polysilicone-15 for use in cosmetics and hair products at up to 10%.

Summary/recommendation

The sponsor has provided an *in vivo* genotoxicity study as requested, which shows that polysilicone-15 did not induce micronuclei in mouse bone marrow. Further kinetic analysis (*in silico*) of the benzylidene malonate groups attached to polysiloxane backbone was provided, which indicated a very low likelihood of potential hazard associated with degradation of the parent molecule and side groups. The sponsor did not submit a 3 month dermal toxicity study, but provided information on short-term dermal exposure in humans, an expanded history of use in products and an expert report highlighting positive aspects of polysilicone-15 use.

Polysilicone-15 has no adverse effects in *in vitro* (previously assessed) and *in vivo* assays assessing mutagenic/genotoxic potential.

Characterisation of its metabolism focused on *in silico* analysis, which indicated a very low likelihood of hazardous degradation products.

Repeat-dose dermal toxicity was not provided, however, likelihood of polyisilcone-15 being carcinogenic was lessened by negative *in vivo* genotoxicity results. Limited human data on dermal application showed a protective effect against changes associated with possible adverse cellular changes. History of use in EU shows >55000 kg used in products over 5 years, with no reported adverse effects.

The committee's opinion is requested.

Submitted data/reports

Micronucleus assay in bone marrow cells of the mouse with Parsol SLX (s47F s47F; study no. 927700; s47F; May 2006; GLP/QA-yes).

The purpose of this study was to determine whether Parsol SLX had the potential to induce the formation of micronuclei in polychromatic erythrocytes (PCE) extracted from the bone marrow of NMRI mice. Groups of mice (5/sex/dose) were exposed to Parsol SLX and control agents prior to assessment of the occurrence of micronuclei in PCE's from bone marrow; at least 2000 PCE's/animal were evaluated for the presence of micronuclei. The test material was administered as a single oral dose (volume 10mL/kg) to groups receiving either 500, 1000 or 2000 mg/kg, with bone marrow collection conducted at 24 hours (500, 1000 and 2000 mg/kg groups) and 48 hours (2000 mg/kg only) post-dosing. Doses used in this study had been established in a pre-study range-finding assay. Assessment of possible cytotoxicity was determined using the PCE to normochromatic erythrocyte (NCE) ratio as an index. Also, the PCE numbers in treated groups and vehicle controls were compared. Corn oil was used as the vehicle (given alone in control group) to deliver the test material and a positive control group was given 40 mg/kg cyclophosphamide PO.

Cytotoxicity was not evident, with PCE/NCE ratios similar across treatment and vehicle control groups, as well as no significant change in the average values for PCE's in treatment groups and controls. Animals survived dosing with limited clinical signs of toxicity; these consisted of ruffled fur at the highest dose and no signs at either lower dose. Analysis of the test formulations showed that all concentrations correlated with the intended value, with recoveries between 98.6 to 102.6%.

Results for the micronucleus assay are summarised in the following table:

Test group	Dose mg/kg	Sampling time	PCE's with micronuclei	Range	PCE/2000 erythrocytes
Vehicle	0	24	0.115%	1-4	1140
Test item	500	24	0.080%	0-4	1105
Test item	1000	24	0.080%	0-3	1149
Test item	2000	24	0.095%	1-5	1142
Positive control	40	24	3.505%	45-104	1111
Test item	2000	48	0.120%	0-5	1124

Statistical analysis of the data revealed that none of the test item groups showed any significant variation from the vehicle, while the positive control was significantly ($p<0.0001$) different to the vehicle control.

Overall, a comparison between test groups and the vehicle control showed there was no statistical significant or biologically relevant difference in the rate of formation of micronuclei for any dose or analysis time (24 or 48 hours) used in this study. The incidences of micronuclei induction occurred at a similar frequency and across a similar range in test groups and vehicle controls. Results from the positive control group showed a 30 fold greater induction of micronuclei compared with both the test groups and the vehicle control. Parsol SLX was not clastogenic in this assay.

Polysilicone-15 UV filter; *In silico* safety assessment – metabolism and toxicology s47 [redacted] s47 [redacted] **May 2006;** s47F [redacted] **et al):-** attachment 2.

At the October 2005 meeting of the MEC, concerns were raised by the committee regarding the activity of possible metabolites of polysilicone-15 and the malonate groups attached to the polysiloxane chain. Information in attachment 2 provided details of a further *in silico* investigation of the activity of potential metabolites of polysilicone-15 and the benzylidene malonate groups attached to the polysiloxane chain.

s47 [redacted]. Using METEOR, which uses expert knowledge rules in metabolism to predict the metabolic fate of chemicals based on the chemical structure, the s47 [redacted] were analysed.

s47 [redacted]

s47 [redacted]

Metabolites predicted (by METEOR) to be released from the polysiloxane backbone were subjected to analysis by DEREK (rule-based expert system that predicts the toxicological hazard of chemicals based on substructure). The metabolites nominated as probable were put through DEREK, which searched against the knowledge base for alerts and reasoning rules on the structures. DEREK found no structural alerts, which indicated that there was no hazard associated with the metabolites identified by METEOR.

Although there were no structural alerts for **probable** metabolites, the sponsor included a calculation for the most probable metabolite released from the polysilioxane backbone, s47 [redacted]

s47 [redacted]

Hazard analysis of **plausible** metabolites (low likelihood) using DEREK was conducted.

s47 [redacted]

s47 [redacted]

It was stated that, "the *in silico* analyses for metabolism and toxicity found no toxicity alerts for probable metabolites, only alerts for plausible metabolites; all alerts are over-ruled by *in vivo* data". Also, with the average molecular weight of polysilicone-15 determined to be approximately 6000g/mol very low dermal and oral absorption is predicted. The sponsor concluded that, "the *in silico* metabolism and toxicity evaluation did not reveal any hazards for polysilicone-15, which have not already been assessed by *in vitro* and *in vivo* toxicity tests. Therefore, there is no foreseeable risk for the consumer from polysilicone-15".

It was noted from the original evaluation that identification of possible metabolites of polysilicone-15 was affected by the very low recovery of related material following dermal application and oral administration.

Note: in silico is a term that refers to the evaluation and graphical display of experimentally gained computer data. Probable was defined as, "there is at least one strong argument that the proposition is true and there are no arguments against it". Plausible was defined as, "the weight of evidence supports the proposition".

The detrimental effects of daily sub-erythemal exposure on human skin in vivo can be prevented by a daily-care broad-spectrum sunscreen (s47F [redacted] et al; presented in J. Investigative Dermatology; author's from St John's Institute Dermatology; Guy's Kings and St. Thomas School Medicine; King's College; St Thomas Hospital; s47 [redacted] s47 [redacted] report no. 2500214; Feb. 2006).

Background: In the introduction to this study, a link between UV radiation and damage to the skin was described. An association (based on action-spectra) between non-melanoma skin cancer, photo-aging and human erythema was made to UVB radiation. The similarity in action spectra was indicative of a common biological mechanism (chromophore), which was likely to be DNA. Recent work in molecular epidemiology supports a function of UVB radiation in non-melanoma skin cancers in humans. IARC (International Agency for Research on Cancer) have stated (2001) that, "there is limited evidence in humans for a cancer preventative effect of the topical use of sunscreen formulations against squamous-cell carcinoma of the skin. IARC also found that, "there is inadequate evidence for any preventive effects against basal cell carcinoma and malignant melanoma". It was acknowledged that skin cancer is a multi-factorial process that involves DNA photo-damage, inadequate DNA repair, probable failure of apoptosis, mutation, clonal expansion of mutated cells and UVR-induced immunosuppression. An initial effect of UV radiation of human skin is erythema (sunburn), which can lead to damage described above. Studies investigating (*in vivo*, in humans) skin damage were suggested to be based on single UV exposures (acute studies), which may not be representative of effects following repeated-suberythemal exposure including adaptive changes beyond obvious tanning. This study examined the effects of repeated-suberythemal exposure to solar simulated radiation in the presence and absence of sunscreen (low SPF).

In this study a panel of subjects were exposed to repeated sub-erythemal doses of solar simulated radiation. The effects of 11 consecutive daily sub-erythemal (0.52-0.62 MED)

exposures to solar simulated radiation (SSR) in individuals (n=6) with sun-sensitive type 1 and 2 skin, as well as the protection offered by a low SPF broad-spectrum sunscreen were investigated. The sunscreen formulation used contained low levels of polysilicone-15 (6%) and butyl methoxydibenzoylmethane (2%), which had a SPF of 7.5 and a combined absorption maxima range from approximately 315 to 360 nm. The study included a vehicle control and non-irradiation control sites for comparison. Erythema (erythemal index EI) was assessed daily, while indices of immune of molecular and cellular damage were assessed on days 5 and 11, as well as 24 hours after (day 12) the last irradiation. In the unprotected irradiation situation, evidence of induced erythema, DNA photo-damage (thymine dimers), p53 protein expression, and Langerhans cell depletion was revealed.

Results: The first end point assessed was erythema, with data showing no discernable erythema in the sunscreen group (EI range 18-28) over the exposure period, while erythema was observable in the vehicle control group (EI range 20-95) from approximately day 5 through to the end of the exposure period. The mean level of erythema displayed by the vehicle control site was normally equivalent to approximately 1 MED with definite borders. It was stated that the eye can detect erythema at an EI of approximately 50.

Measurement of the presence of thymine dimers (TD) at vehicle control and sunscreen treated sites showed less DNA (TD presence) at the sunscreen treated site against background damage threshold. Protection offered was significant ($p<0.01$) across the subjects in the study.

Determination (counts of positive cells, staining) of the effects on p53 protein expression throughout the epidermis was displayed in graphical form, which showed that expression was very low at all sites treated with a sunscreen. Indices of p53 expression increased steadily at vehicle application sites from approximately 20 units at day 5 to 45 units at day 12 (day 11 = 30 units), while p53 expression stayed below 5 units on sunscreen treated sites on days, 5, 11 and 12. The amount of p53 expression at sunscreen protected sites was marginally greater than a non-irradiated site. It was noted that the vehicle sites had high expression (approximate minimum increase 8 fold) at all time points, high variation/fluctuation resulted in the day 11 increase in p53 being the only time the sunscreen showed significant protection.

An indicator for apoptosis (Bcl-2 protein) was not changed suggesting that apparent damage (changes in thymine dimers for DNA photo-damage and p53 expression) did not trigger an apoptotic response. Also, no sunburn cells were detected in any of the sections examined.

Langerhans cells (LC) are the skin antigen presenting cells and are known to be sensitive to UV radiation. Data showed a trend in the marker (CD1A) for LC, which indicated a time-dependent depletion at the vehicle irradiated site when compared to no irradiation. Data for the sunscreen protected site showed no difference in LC when compared to the non-irradiated site.

It was stated that epidermal proliferation showed a large degree of variation amongst the individuals in the study. The mean number of cell layers was not affected by any of the test situations thus not allowing a comment to be made on the value of the sunscreen on this parameter.

Results indicated that repeated daily sub-erythemal exposure to UVR on type1/2 skin lead to molecular and cellular damage. Apoptosis and epidermal thickening were not stimulated by

the observed changes. Overall, it was shown that the sunscreen (included Parsol SLX) provided significant protection against the adverse effects of solar radiation. It was noted that no sunburn cells and epidermal thickening were observed under any study condition (including vehicle control). The usefulness of sunscreen use in cancer reduction or prevention is unclear, but it is apparent that sunscreen can inhibit the changes that may be associated with the development of skin cancer. It was suggested that daily care with a sunscreen may offer protection against non-intentional solar exposure.

Assessment of photoprotection provided by daily Sunscreen use (s47F and s47F s47F s47 ; study HLR 01)

It would appear that this report is continuous with the previous study. In a preface to the previous report it was stated that, "this work is to be submitted for publication; 2 documents are presented. The first entitled, **T**he detrimental effects of daily sub-erythemal exposure on human skin in vivo can be prevented by a daily-care broad-spectrum sunscreen, is the form in which the work will be submitted for publication; it contains a detailed discussion of the results, but does not include all of the data. The second entitled, Assessment of photoprotection provided by Daily Sunscreen Use, lacks the detailed discussion of the results, but it includes all of the data". Both reports were considered and assessed in the preceding text.

Expert report of polysilicone-15 (s47F , 2006).

In this expert report s47F addresses a number issues relating to the formulation of UV filters in sunscreens (attachment 3). s47F supports the following issues with published articles, and company studies and data.

In his expert report, s47F provides comment on areas related to the use and approval of polysilicone-15. He states that, there is a need for better UV filters (including UVB) to improve compliance (cosmetic acceptability) and protection offered by sunscreens against the adverse effects of UV radiation; he states there is a need to develop more stable UV filters, with reduced capacity to penetrate skin; he discusses the stability of UV filters in formulation; he addresses the adverse reactions profile of UV filters; he highlights the extent to which polysilicone-15 is used in the European market.

The report emphasises the damaging and pervasive nature of UV radiation (UVR) with the most apparent effect of excessive UVR manifest as sunburn, which is due to UVB (85% at 290-320nm), UVAII (9% at 320-340nm) and UVAI (6% at 340-400nm). A link between UVB exposure and non-melanoma skin cancer is made based on similar action spectra and work showing a dose-related reduction in the incidence of solar keratoses (marker for squamous cell carcinoma- SCC) with sunscreen use (SPF 15+ for 7 months).

The recent approval history of UVA filters by the MEC/TGA was mentioned by the author, along with the fact that no new UVB filters have been approved. The author conducted a sample survey (products with SPF15 or greater from 7 companies) to support his contention of usage patterns of UV filters. His survey showed that of the organic UVB filters octyl methoxycinnamate (42 products) was most used followed by methylbezylidene camphor (26 products), octocrylene (15 products) and octyl triazone (12 products), with other available UVB filters used in less than 5 products. The most frequently used organic UVA filters were butyl methoxydibenzoylmethane (24 products) and oxybenzone (14 products).

He notes that apart from the current inorganic UVB filters, current organic UVB filters show appreciable skin penetration. Information provided by the author indicates that percutaneous absorption for octyl methoxycinnamate (up to 2%), methylbezylidene camphor (up to 1.9%), octyl triazone (0.5-1.5%) and oxybenzone (up to 2%) was greater than that for polysilicone-15 (0.2%). The author noted that none of a series of different vehicles in which polysilicone-15 was tested enhanced total penetration (stripped skin + receptor fluid) beyond 0.6%, while octyl methoxycinnamate absorption varied from 1.6% to 5.4% with different vehicles.

Also, the popular UVB absorber, octyl methoxycinnamate, shows photo-instability and this means that it is less efficient than expected and may release photo-degradation products (unassessed). The photo-instability of octyl methoxycinnamate is enhanced in the presence of butyl methoxydibenzoylmethane which leads to the inclusion of other UV actives to compensate for degradation. Adverse reactions to UV actives (or breakdown products) have been documented and a summary of these findings was presented in the report. Typically dermal reactions such as skin sensitisation (allergies) and photo-patch (photo-sensitisation) testing to octyl methoxycinnamate have been described as occurring at a low frequency. Recorded contact allergy to octocrylene was described in a published article as a result of passive transfer in a cosmetic (sunscreen). Oxybenzone has been described as a common dermal allergen in a number of published articles summarised by the author. The use of butyl methoxydibenzoylmethane has been recorded as resulting in both contact and photo-contact sensitivity to sunscreens, as has methylbezylidene camphor and octyl triazone. The author concluded by stating that although allergic and photo-allergic reactions to sunscreen actives are not common, they do occur on a regular basis. These adverse reactions are a strong indication that current organic UV absorbers do penetrate the skin to a sufficient extent to evoke a reaction. He also stated that there appears to be a need for a UVB absorber with negligible ability to reach viable responsive tissue.

The expert report included information provided by s47 on the effect of substituting polysilicone-15 for octyl methoyxcinnamate in two separate formulations (all other ingredients stayed the same) with SPF values of approximately 20 and the low 40s. There was no significant difference (20 vs 21; 44 vs 41) in SPF value for the formulations, which were providing high protection (high SPF). Tabulated information on possible synergistic effects of polysilicone-15 was discussed in the report. Tabulated data showed significantly improved SPF with polysilicone-15 present, which may have been due to photo-stabilisation. A specific photo-stabilisation study examined the effect of high UVR (10 minimum erythemal dose, MED) on an emulsion containing 2% butyl methoxydibenzoylmethane in the presence of varying concentrations of polysilicone-15. At 10 MED 2% butyl methoxydibenzoylmethane greater than 80% of butyl methoxydibenzoylmethane was lost; at 2% butyl methoxydibenzoylmethane and 3% polysilicone-15 approximately 25% of the 2% butyl methoxydibenzoylmethane was lost; at 2% butyl methoxydibenzoylmethane and 7% polysilicone-15 approximately 10% of the 2% butyl methoxydibenzoylmethane was lost. Polysilicone-15 displayed a dose-related protective effect on the photo-degradation of butyl methoxydibenzoylmethane.

The report provided information of the usefulness of using sunscreen (SPF 15+) against squamous cell carcinoma and basal cell carcinoma; the information was taken from a 4.5 years study. In this study a comparison was made between groups using the sunscreen 4 times/week compared to a group using the sunscreen 0-2 times/week, indicating more frequent usage is more effective. The report identified compliance (reduced quantity/less

frequent than necessary) as an important factor in the protective behaviour of sunscreens. According to the author an issue affecting compliance is the property of cosmetic acceptability, with the consumers finding greasy or white/grey residues unpleasant and off-putting. It was noted that polysilicone-15 had an exceptionally good cosmetic profile. A description of a test for cosmetic acceptability was presented in the expert report, where agents were subjected to a sensorial assessment on human volunteers. A product made up of combination of polysilicone-15 (3%) plus titanium dioxide (3%) was compared to product containing titanium dioxide (6%), with both products providing protection at a SPF rating of 27-28. Compliance for use of amount of product was greater for the combination product, with volunteers using 29% more providing better skin protection.

The use of inorganic UV filters may be considered adequate, but it should be noted that they are always used in tandem with organic UV filters. The reason for co-formulation of inorganic and organic UV filters is both cost and that they work synergistically together; it is possible to achieve very high SPFs more readily using a combination of organic and inorganic actives than using only organic actives. An additional reason is that inorganic sunscreens can be added to the formulation to achieve the broad spectrum standard of AS2604. There is a place for an organic UVB filter that has very low skin penetration, which is where polysilicone-15 has merit according to the author.

Comment on the history of use of polysilicone-15 stated that it had been now used in Europe since 2001. Between 2001 and 2005 s47 [redacted] over this period of time there has been no adverse effects reported.

Polysilicone-15 was described as easy to formulate because it is a liquid and is readily miscible with other components of the formulation. It was noted that it could be heated to 80ºC for 6 hours without losing activity. In comparison, many other UV filters (oxybenzone, butyl methoxydibenzoylmethane, octyl triazone, methylbenzylidene camphor) are solid at room temperature and are often difficult to dissolve in the formulation. Sunscreen formulations require co-solvents to aid dissolution of these agents and octyl methoyxcinnamate (itself a UV filter) and organic esters are used as co-solvents. Problems with solubility of UV filters can lead to crystallisation of the sunscreen actives and loss of SPF during long-term storage. The author noted that if UV filters are present at concentrations close to their solubility limits this has an effect of increasing the potential of skin penetration. Also, it was described how skin penetration of organic sunscreen actives is formulation dependent, with different excipients/vehicles having the potential to alter physical properties of the actives within the layers of skin.

NICNAS, Full Public Report, Parsol SLX, March 2006 (attachment 4).

Polysilicone-15 was approved by NICNAS for use in skin and hair care end-use products (cosmetics) up to a maximum concentration of 10%. The full public report is included in this submission at attachment 4. Approval by NICNAS appeared to be based on the data set provided to the EU for approval as a UV filter in Europe.

s22 [redacted]
OTCMES, NPM Branch, July 2006

OTC MEDICINES EVALUATION SECTION –TGA
EVALUATION OF A NEW SUNSCREEN ACTIVE

TINOSORB M

2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol

Sponsor: Ciba Specialty Chemicals Pty Ltd
File Number: 98/23613
TGAIN Number: 109719
Prepared by: s22 [redacted]
Report Date: August 2002

Overview of new data:

This report includes the evaluation of new data in support of a re-application for Tinosorb M by the sponsor seeking approval for its use as an UV-A radiation filter. The MEC has previously recommended rejection of this application on the grounds that there were insufficient data to allow an adequate safety assessment of this substance prior to granting approval for its use in listed products (see attachment 1 & 2).

New data consisted of pharmacokinetic and repeat-dose dermal toxicity studies, and a comment/justification addressing carcinogenicity assessment. The new data indicated that both oral and dermal absorption of Tinosorb M was very low in the rat. Absorption was greater via the dermal route when compared to the oral route, but total absorption was <1% for either route. There was no evidence of metabolism, since only one peak (parent) was found at analysis. In the dermal toxicity study (up to 1000 mg/kg for 13 weeks), there was no evidence of systemic toxicity, while limited local damage (linked to occlusive dressing) at the application site was found to be similar in treated animals and controls.

A justification to waive the need for long-term dermal carcinogenicity bioassays was based on studies showing that Tinosorb M was not a mutagen, it did not cause hyper-proliferative skin damage, it was not a photo-mutagen, it was not a skin contact allergen or irritant, it was not photo-toxic, it was photo-stable and it did not fit the profile of known skin carcinogens.

Attachments:

1. MEC minutes from June 1999 for Tinosorb M……..
2. MEC minutes from September 2000 for Tinosorb M……...……..
3. Sponsor's foreign use data file……
4. Sponsor's justification for waiving carcinogenicity bioassay……
5. Evaluation report by s22 [redacted] (1999)…….

Introduction

The sponsor has recently (June 2002) submitted new data and information on an upgraded regulatory status of Tinosorb M in support of a reassessment and possible acceptance for its use in listed sunscreens in Australia.

The MEC had previously considered this application at its meeting of June 1999 (item 4.1). At that time the MEC recommended rejection on the grounds that there were insufficient data

to allow this substance to be approved for use in listed products (see attachment 1). Tinosorb M was again presented (Sept. 2000, item 4.1) to the MEC following the submission of additional data and an EU regulatory assessment conducted by the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP). It was again rejected on the basis that the information provided by the sponsor was insufficient to establish the safety of the substance (attachment 2).

Information on the identity of Tinosorb M was also provided. Its CAS number is 103597-45-1 and its ELINCS number is 403-800-1. Tinosorb M is a dispersion of micro-fine particles with a unique chemical structure making it an effective absorber of UV radiation in the energy range defined by the UV-A wavelengths (320-400 nm). Tinosorb M and MBBT [2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol] are names that identify the same substance throughout this report. It was noted that Tinosorb M has been identified as MBBT in some of the supporting studies.

Regulatory status

A European Community Directive (2000/6/EC) of February 2000 stated that, within the concentration limits and under the conditions adopted by the cosmetic industry for its use as a UV filter for sunscreen products, 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol (Tinosorb M) is not likely to produce harmful effects for the health of users. Therefore, it may be included in the list in Part I of Annex VII.

Part I of Annex VII has a reference (no. a/23) to Tinosorb M indicating that it can be used at a concentration of up to 10% in sunscreen products.

It has now been two and a half years since Tinosorb M was approved in the EU. Its acceptance has extended to South America, with its accepted use expected in some Asian countries in the near future. European countries using Tinosorb M include France, Germany, Switzerland, Spain and Greece, while it is also used in Brazil. A total of 35 products are currently marketed in these 6 countries. Some products containing Tinosorb M are Avene 60 (Tinosorb M, octyl methoxycinnamate, zinc oxide), Minesol 60 (Tinosorb M, octocyclene, octyl methoxycinnamate, titanium dioxide) and Photoclean Max 100 (Tinosorb M, 4-methylenzylidene camphor, butyl methoxydibenzoylmethene). These products claim SPF values of 60-100 as indicated in their names. Supporting documentation (see attachment 3) indicates that to date, there have been no adverse drug experiences filed for any sunscreen products containing Tinosorb M. These data relate to a volume of Tinosorb M sold in France of 161.6 tons since approval, and 12.8 tons in the other 5 countries; all as OTC sales.

European regulatory report (summary)

The sponsor submitted to the TGA a copy of the assessment (COLIPA no. S79) of Tinosorb M, which was conducted by the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP). In the process of their assessment the SCCNFP is required to determine whether a compound is safe for use in cosmetic products, and whether there is the need to impose restrictions or conditions for its use in cosmetic products. The SCCNFP was of the opinion that 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol (Tinosorb M) is safe for use in cosmetic products as a UV light absorber at a maximum concentration of 10%.

The data package evaluated by the SCCNFP was the same as that submitted to the TGA (includes new studies in this report and studies described in a previous report by A. s22 [redacted] of the CPA, attachment 5) for assessment. Consideration of this data was the basis for the approval for the use of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol in cosmetics in the EEC.

In summary, the SCCNFP noted that there were no special signs of toxicity observed in acute oral and dermal (no deaths at max. dose 2 g/kg) toxicity studies. There was no evidence of toxic effects on either the in-life or pathomorphological parameters in rats dosed orally with 1000 mg/kg/day for 90 days. The test compound was not phototoxic or a photosensitiser in guinea pigs. No developmental toxicity was observed in rats at orally doses up to 1000 mg/kg. The overall penetration of the test substance through the skin is very low and is taken into account for the calculation of the margin of safety. The test substance was a non-irritant (according to 83/467/EEC) on skin, and in the eye and on mucous membrane (CPA/TGA report stated that a slight skin and eye irritation was present). The test substance was not genotoxic/mutagenic (including photo-mutagenic) in the presence or absence of metabolic activation in bacterial cells and mammalian cells (Chinese hamster ovary cells). Clinical data showed that the test substance was not photo-allergenic or -toxic in human volunteers.

A calculation of safety margin by the SCCNFP used the following data to arrive at value of 476. Maximum amount of ingredient applied at 1800 mg, at an absorption rate of 7%, with a NEL of 1000 mg/kg in a 60 kg human.

Systemic exposure: 1800 x 7%/60 kg = 2.1 mg/kg

Safety margin: 1000/2.1 = **476**.

It was noted that a re-calculation of the safety margin assuming that all the product left in the viable epidermis + dermis is systemically available (22%), would result in a safety margin of 151, which was still acceptable to the SCCNFP.

For comparison, the TGA/CPA evaluator estimated that up to 2.9 g of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol could be applied if a male with a body surface area of 18000 cm^2 was to apply sunscreen at 2 mg/kg (based on the Australian standard for determining SPF). This appears an overestimation of dose applied since it takes into account the entire average body surface area. However, the evaluator accepted that 7% (based on *in vitro* data) was the amount absorbed systemically following dermal application and that the NEL for the sub-chronic toxicity was 1000 mg/kg. Based on these values the difference between the human exposure dose and the NEL in the animal study was **296** times.

ASSESSMENT

The minutes of MEC (meeting 3/6/199, item 4.1) indicated that the committee agreed with the evaluator (s22 [redacted], CPA) that there were insufficient data to support acceptance of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol. It was noted that this substance had not been approved for use in/on humans for any purpose to date. Furthermore, there was a lack of *in vivo* kinetic data (not been addressed) and limited genotoxicity data, with no reproductive or carcinogenicity assessment (no data had been presented in these areas). The committee also noted that since this substance was a UVA blocker, it would need to be in a formulation with a UVB blocker; safety concerns were

raised over formulation (interaction) with UVB and excipient substances. Additional concerns were identified by MEC, following presentation of an upgraded report (additional data supplied by sponsor) in September 2000, that focused on kinetic data required to fully understand the significance of the oral and dermal toxicity studies. The preceding issues have been addressed in the following assessment.

Regulatory status

In response to MEC concerns the sponsor has provided details of approval being granted (Feb. 2000) in the EEC for its use as a sunscreen at a concentration of up to 10% in a product. It has now (September 2002) been two and a half years since Tinosorb M was approved in the EU. Its acceptance has extended to South America, with its accepted use expected in Asia in the near future. European countries using Tinosorb M include France, Germany, Switzerland, Spain and Greece, while it is also used in Brazil. A total of 35 products are currently marketed in these 6 countries. Supporting documentation (see attachment 3) indicates that to date, there have been no adverse drug experiences filed for any sunscreen products containing Tinosorb M. These data relate to a volume of Tinosorb M sold in France of 161.6 tons since approval, and 12.8 tons in the other 5 countries; all as OTC sales.

Kinetic interpretation

The issue of kinetic data regarding dermal penetration was addressed in an assessment provided by the sponsor. Based on the *in vitro* assay of percutaneous absorption of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol in a standard sunscreen formulation, a safety margin of approximately 170 was estimated (this varied from original estimate).

The MEC questioned whether/how damaged skin (abraded or burnt) would affect absorption of Tinosorb M? A company statement in reply noted that if damaged skin caused a doubling of the dermal absorption rate then, "the margin of safety would be 85, which is still very close to the acceptable safety margin of 100". No data was presented that actually measures the effect of damaged skin on absorption and the example of a doubling is a hypothetical situation. However, there were no *in vivo* data on absorption of the test material. The sponsor provided comments on methodological as well as interpretation problems when examining absorption in such situations. Qualification of these considerations included issues of ethical (human studies) concerns and the need to examine a condition of altered barriers to absorption; stated oral studies test when almost no barrier was presented. However, recent studies examining *in vivo* oral absorption in rats indicated that the GIT wall/lining was a greater barrier to the absorption of Tinosorb M than the skin.

The MEC asked about the need for *in vivo* kinetic data. In reply, the sponsor justified the lack of *in vivo* data on the grounds that it is not required according to COLIPA guidelines, and the *in vitro* study was accepted by SCCNFP. Included in the data package supporting this application was a study (using testosterone) validating the correlation between *in vivo* and *in vitro* dermal absorption. The sponsor concluded that they believe the *in vitro* data gives a representative estimate of the bioavailable amount of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol after dermal application.

However, the MEC indicated that they required toxicokinetic (including data on possible metabolism of Tinosorb M) data to enable a full assessment of the oral and dermal toxicity studies in species used in these toxicity studies.

The sponsor authorised the conduct of studies examining the pharmacokinetics of Tinosorb M in the rat, as well as a 3 months dermal toxicity study in rats, which incorporated toxicokinetic analysis. In the pharmacokinetics study, orally administered radiolabelled Tinosorb M was rapidly and extensively excretion in the faeces (up to 97% within 72 hours). Analysis of the faeces revealed only one fraction, which was the parent compound indicating an absence of metabolism in the GIT. Urinary excretion of the radiolabel was <0.01% of the administered dose in both sexes of the rat strain used in the study. Analysis of the urine for metabolites was impossible due to the extremely low recovery. Such low absorption via the oral route places the results of the oral toxicity study into perspective, with probably less than 1 mg/kg/day absorbed from an administered dose of 1000 mg/kg/day (rat 3 months oral toxicity study). This finding would apply to any study where Tinosorb M was administered orally, and identifies the futility of carrying out oral studies of any kind using Tinosorb M.

Absorption (systemic exposure) following dermal application of radiolabelled Tinosorb M was low, amounting to between 0.2 and 0.8% in the rat. Less than 0.01% of the dose was excreted in the urine (similar to oral route) and less than 0.05% in the faeces at any time point. Approximately 97% of the test material remained on the skin after the treatment period. Metabolite profiling was not possible due to the extremely low amount passed in the urine and faeces. It was apparent that more of the radiolabelled Tinosorb M was absorbed via the dermal when compared to the oral route. Toxicokinetic data were generated from a 3 months repeat dose dermal toxicity study. Unfortunately, there was clear evidence (control samples with higher levels than test samples) that the test and control samples were contaminated with Tinosorb M and the results were deemed invalid.

The *in vivo* kinetic enables a reassessment of estimates of relative exposure to be carried out. Firstly, the SCCNFP used a figure of 7% as the likely percutaneous absorption based on *in vitro* data, but the *in vivo* animal data shows a much lower figure of <1% percutaneous. Unfortunately, there are no data to allow a rational comparison of the results obtained for dermal absorption in humans and rats. However, a value of 7% would appear to be relatively high given the chemical nature (size, partition coefficient, low lipid solubility of 0.17%) of Tinosorb M. Lower percutaneous absorption, approaching the *in vivo* figure (<1%) seen in rats, would increase the estimated safety margin.

Interestingly, the SCCNFP estimate of systemic exposure of 2.1 mg/kg (using 7% absorption, see p3) in humans is approximately 4 times lower than the estimated exposure of approximately 8 mg/kg for the 3 months dermal toxicity. Even the oral toxicity studies (repeat dose and reproductive toxicity) have similar systemic exposure levels to the SCCNFP estimate of systemic exposure in humans. Therefore, oral and dermal toxicity studies in animals showed no evidence of toxicity at similar and higher levels of systemic exposure estimated to occur in humans. It is possible that the oral toxicity studies are not totally meaningless when considering the safe use of Tinosorb M.

Repeat dose toxicity data

Since administration via the dermal route provided greater systemic exposure, the sponsor carried out a 3 months dermal toxicity study in rats, with exposure to Tinosorb M (150, 450 or 1000 mg/kg/day) for 6 hours daily under a semi-occlusive dressing. There was no evidence of systemic toxicity over the duration of the study and through a drug-free recovery period. Local irritation was evident, but this occurred to a similar degree in treated animals and controls at all dose levels, which led to the conclusion that the irritation was to due to mechanical irritation from the dressing. A NEL of 1000 mg/kg/day was established for Tinosorb M applied dermally. An oral toxicity study had previously been submitted and evaluated. In this study doses of up to 1000 mg/kg/day Tinosorb M in rats had no adverse effects (NEL 1000 mg/kg/day).

Interaction with other UV absorbers

The response from the sponsor focused on high photostability of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol at simulated sunlight exposures up to an equivalent dose of 50 MED (50 x minimal erythmetic dose). It was shown that there was a 99% and 98.3% recovery of the test material at 20 and 50 MED, respectively. Furthermore, it was shown that 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol retained its stability when formulated in sunscreen products with UV-B absorbers and excipients. It was found to be the most stable UV absorber and improved the stability of other UV absorbers.

The sponsor noted that the MEC questioned whether dermal absorption would be altered by other components of a sunscreen. The sponsor stated that the *in vitro* testing of percutaneous absorption of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol was carried out using full sunscreen formulations, with estimates of absorption based on expected exposure conditions.

Dermal irritation

Dermal irritation in the animal (in rabbit) study was addressed by a comment from the sponsor. It was stated that, "it is our assessment that 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol in sunscreen at 10% will not cause chemical irritation of the skin". The reason behind this statement is the claim that in the rabbit study the dermal exposure to the test substance was 5 times higher than likely exposure following an estimated human dose. It was also suggested that rabbit skin is more sensitive to irritation than humans, so the incidence of barely perceptible reddening in rabbits is not a clear prediction of irritation in humans.

It should be noted that during the phototoxicity and photoallergy studies in humans the incidence of irritation (predominantly erythema during induction phase) was similar in saline controls and subjects treated with 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol. The incidence of irritation could have been due to the occlusive dressing; irritation is a common in assays where occlusive dressings are used.

Reproductive effects

The potential teratogenic activity of 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol was examined in a reproductive toxicity study in rats. The test substance was not teratogenic up to the maximum dose used of 1000 mg/kg/day PO. This level of investigation into the reproductive toxicity of a new test substance would be considered inadequate by DSEB standards, with fertility and peri/post natal studies required, as well as a second developmental study in a species other than a rodent (usually in rabbit). However, studies investigating these reproductive end-points using the oral route would be futile due to extremely low absorption from the GIT. Furthermore, it is doubtful that dermal application of realistic dose levels would generate adequate systemic exposure to test reproductive toxicity.

In the repeat dose dermal toxicity study, there were no adverse effects on the oestrus cycle of the female rats, or on hormones such as testosterone, oestradiol, TSH, FSH and LH. In studies such as *in vitro* genotoxicity assays, diffusion across barriers (from GIT or through skin) was not an issue (except into cell) and results showed the test substance had no adverse effects on genetic material. The preceding information may assist in understanding the potential reproductive toxicity of Tinosorb M, since the oral reproductive toxicity study in rats had a limitation based on low systemic exposure.

Genotoxicity and carcinogenicity

Genotoxicity was examined in bacterial and mammalian cell systems. Previously, 3 *in vitro* studies examining reverse mutation in bacterial cells (2 studies) and chromosomal aberrations in mammalian cells (1 study in Chinese hamster ovary cells [CHO]) were evaluated and found to be negative. One of the assays in bacterial cells examined possible photomutagenic activity, which was found to be negative. A recently submitted study using mammalian cells (chromosomal aberration assay in CHO cells) also found that the test substance was not photomutagenic. DSEB requirements are flexible, but would normally expect an *in vivo* assay (possibly micronucleus assay) to provide a more complete assessment of potential genotoxic activity of a new substance. An *in vivo* assay in the testing process does not appear to be appropriate based on the limited exposure achievable through various routes (primarily oral) of administration.

The sponsor has noted that the lack of genotoxicity as well as the lack of systemic toxicity (no cell toxicity or regenerative hyperplasia) up to a dose level of 1000 mg/kg/day for 3 months supports a lack of carcinogenic potential. The 3 months toxicity study is a relatively short period for the development of non-neoplastic changes that could be initial stages of future neoplastic events. However, the relevance of these findings are questionable since pharmacokinetic data showed that systemic exposure following oral administration was likely to be <0.1% of the dose (approx. 1 mg/kg).

The sponsor has included a justification to waive long-term carcinogenicity bioassays in the most recent data package (attachment 4). The following points summaries the sponsor justification:

1.Tinosorb M is not genotoxic or aneugenic in mutagenicity assays.
2.Tinosorb M did not induce dermal acanthosis or other hyperproliferative dermal changes in rats after repeated dermal dosing.

3.Tinosorb M is stable to UV light and does not generate photo-metabolites or breakdown products.
4.Tinosorb M is not a skin contact allergen in the absence or presence of UV irradiation and it does not cause phototoxic skin reactions.
5.Tinosorb M does not have the profile of known skin carcinogens and will not be a dermal carcinogen.
6.Dermal carcinogenicity testing of Tinosorb M is not necessary to further substantiate this UV filter as safe for use in listable sunscreen products in Australia.

Points 1 to 4 were covered and accepted as valid in studies submitted in the application for approval of Tinosorb M. The sponsor provided references (published refereed journal articles and monographs from International Regulatory bodies, eg. IARC, FDA-[NTP]) in support of point 5. It was noted that 44 chemical substances (mixtures and medical treatments) are listed as known human carcinogens and among these 10 are known to induce skin cancer in humans. These 10 agents (car tars, creosotes, soots, mineral oils and tars) were shown to be either genotoxic (9/10) or immunosuppressive (2/10). These agents generally contain polycylic aromatic hydrocarbons. Furthermore, the sponsor highlighted that the most notable skin carcinogen is solar radiation, which is directly genotoxic and immunosuppressive. The sponsor then highlighted the findings that Tinosorb M was not genotoxic or clastogenic when exposed to UV radiation (not photo-genotoxic), thus reinforcing that Tinosorb M did not conform to the likely profile of a skin carcinogen. The sponsor concluded that Tinosorb M will not be a dermal carcinogen in rodents or humans and further bioassays were not necessary.

The sponsor also acknowledged there were non-genotoxic mechanisms of skin cancer. “Skin tumour promoters have been known to cause sustained dermal hyperplasia (acanthosis) in mouse skin models. Chronic skin irritation and inflammation, indicated by histological observation of hyperkeratosis and minimal to mild acanthosis could also be relevant to topically applied substances such as UV filters in sunscreens. Several studies in rodents indicate that chronic dermal dosing with the chemical benzoyl peroxide, benzethonium chloride, triethanolamine or oleic acid diethanolamine condensate caused macroscopically and microscopically diagnostic signs of chronic irritation and inflammation, but skin cancer did not occur. These findings strongly support the position that non-genotoxic or other indirect mechanisms of carcinogenicity are not simple and relevant correlates for skin carcinogenesis”.

The sponsor linked this background information with the findings that Tinosorb M was not a skin irritant at 1000 mg/kg/day delivered over 90 days. Results from this study indicated that Tinosorb M did not cause hyperkeratosis or acanthosis, while there was no evidence of systemic toxicity or hyperplasia of any tissues/organs. Tinosorb M did not cause skin sensitisation (immuno response) and was not phototoxic. Clinical trials found that Tinosorb M was not phototoxic or photosensitising in humans.

Overall, the sponsor has put forward a rational approach for waiving the need for long-term dermal carcinogenicity bioassays.

RECOMMENDATION

Recently submitted studies have helped clarified a number of issues that had been raised by the MEC. Pharmacokinetic studies have shown that Tinosorb M does not undergo metabolism (faecal analysis) and absorption is extremely low (<0.1%) following oral dosing in rats. Absorption following dermal application to rats was also very low (<0.8%), with <0.01% of dose excreted in the urine and <0.05% in the faeces. These results give an appreciation of the oral toxicity studies (further oral studies would be meaningless unless at much higher doses), indicating that the systemic exposure to Tinosorb M was likely to be very low (approx. 1 mg/kg/day for a 1000 mg/kg/day dose). A total lack of toxicity at 1000 mg/kg/day PO was due to poor absorption from the gastrointestinal tract.

The sponsor conducted a repeat dose dermal toxicity study using the same (1000 mg/kg/day, in rats for 3 months) parameters as used in the oral study. Analysis revealed no evidence of toxicity following daily dermal application (for 6 hours) of Tinosorb M over a 3 months period. The kinetic study provided results that indicated dermal application at up to 1000 mg/kg/day would probably resulted in less than 8 mg/kg/day systemic exposure to the test material. A value of 8 mg/kg (no toxicity observed) is 4 times greater than the SCCNFP estimate of systemic exposure in humans.

At this level of exposure there were no adverse effects on the oestrus cycle of the female rats, or on hormones such as testosterone, oestradiol, TSH, FSH and LH. In studies such as *in vitro* genotoxicity assays, diffusion across barriers (from GIT or through skin) was not an issue (except into cell) and results showed the test substance had no adverse effects on genetic material. The preceding information may assist in understanding the potential reproductive toxicity of Tinosorb M, since the oral reproductive toxicity study in rats had a limitation based on low systemic exposure. However, the systemic exposure in the oral reproductive toxicity would approximate to an estimated human systemic exposure described in the SCCNFP calculation.

Overall, there has been no evidence of overt toxicity in any study presented for evaluation. Limitations of the oral toxicity studies, and to a lesser extent a dermal study, revolve around limited systemic exposure and how that relates to human exposure. In *in vivo* studies on human skin, Tinosorb M was not phototoxic or an irritant, and it was not a photosensitiser or sensitiser. Tinosorb M is a UVA filter, which was stable when combined with UVB filters. Tinosorb M was not metabolised in rat faeces, while insufficient amounts could be collected from urine or plasma for metabolite profiling.

A sponsor justification to waive long-term carcinogenicity bioassays incorporated data from submitted studies and published data. Tinosorb M was not genotoxic, it did not adversely affect endocrine function in rats, and it was not irritant, phototoxic or photosensitising in humans. Furthermore, Tinosorb M did not fit the profile of known skin carcinogens.

Market-place use of Tinosorb M in sunscreen products for the past two and a half years has not resulted in any reports of adverse effects. Although examination of the potential toxicity profile of Tinosorb M has been limited by its pharmacokinetic properties, a lack of overt toxicity (at similar and higher systemic exposures than expected in humans) is suggestive that its use in sunscreens at concentrations up to 10% would not create a health hazard.

The advice of the committee is requested.

New Data

(submitted July 2002)

Pharmacokinetics

Dermal/oral kinetics

Methylene-bis-benzotriazolyl tetramethylbutyl phenol (CGF-C2089; Tinosorb-M): metabolic fate following oral administration or *in vivo* dermal application in the rat; Central Toxicology Laboratory UK, for Ciba Specialty Chemicals Inc; study no. UR0687; June 2002; R. C. Silcock; GLP/QA-yes.

In summary, the oral and dermal absorption of radio-labelled Tinosorb-M was investigated in the male Alpk:AP_fSD (Wistar-derived) rat. This strain of rat was the same as used in previous toxicology studies with Tinosorb M in these laboratories. In the dermal assay, two similar formulations containing different concentrations (0.2% and 10%) of Tinosorb-M were tested. A measured volume (100 μL) of each formulation was applied to a prepared (shaved) section of skin (10 cm^2), with the applied doses equating to 0.2 mg and 10 mg of Tinosorb-M per rat. It was stated that the amount applied was approximately equivalent to potential human exposure when using sunscreens containing Tinosorb-M (under normal conditions). The degree of oral absorption was studied in both female and male rats given a single dose of 50 mg labelled Tinosorb-M.

The test substance Tinosorb M was supplied to the testing laboratory (CTL) by Ciba Specialty Chemicals as a solid (batch no. 001719BO, 99.6% pure). The radiolabel (1.776 GBq/mMole) was positioned on a carbon bridging between two benzene rings on symmetrical segments of the molecule. The vehicle for the oral preparation was 0.5% CMC/0.1% polysorbate 80 in water. This vehicle was used to maintain a homogeneous suspension of the water-insoluble test substance for oral administration. The preparation used for dermal application was designed to simulate commercial formulations and included plantacare 2000, propylene glycol, xanthan gum and water. It was noted that there were no observable adverse effects in any animal treated via either the oral or dermal route.

In the assessment of oral absorption, Alpk:AP_fSD (Wistar-derived) rat (4/sex) were given a single oral dose of 50 mg labelled Tinosorb-M prior to measurement of radioactivity in urine and faeces over a 3 day period. After this period, the animals were sacrificed and residual radioactivity was measured in blood, selected tissues and the carcass. In addition, another group of rats (9/sex) given the same dose had samples of blood taken for the measurement of radioactivity in blood and plasma over a 24 hours time course.

In the assessment of absorption following dermal application of Tinosorb-M, 32 rats (4/time point) were each given a single dermal dose of either 0.2 or 10 mg of labelled Tinosorb-M. Following treatment the application sites were protected from animals licking the material by use of a non-occlusive system (O-rings and gauze cover). Animals were individually housed in metabolism cages. After 6 hours exposure, surface contact with the test material was discontinued through the use of a washing procedure at the application sites. Collection of tissue samples (skin, urine, blood, faeces, etc) was carried out at intervals from 6 hours onwards (6, 24, 72 and 120 hours post-treatment). Collection of samples included removal of unabsorbed residual test material using a tape-stripping technique to remove the stratum

corneum. All samples, including selected tissues and residual carcasses were analysed for radioactivity.

The results showed that following a single oral dose of 50 mg Tinosorb-M, excretion of radioactivity was rapid and extensive in both sexes of rats. Urinary excretion of the radiolabel was measured at <0.01% of the administered dose in both sexes, while faecal excretion accounted for 96%-97% in both sexes over 72 hours; 80%-92% was found in the faeces within the first 24 hours.. Analysis (HPLC-mass spectroscopy) of the faeces revealed only one fraction, which was found to be the parent compound Tinosorb-M. Analysis of tissues (other than faeces) for metabolites of Tinosorb-M was not possible due to the small amounts of radiolabel/sample recovered. This indicates that there was no metabolism of the parent material during its passage through the GIT.

Residues in excised tissues were determined at <0.01% for all tissues examined, and were not associated with any specific tissues or organs. Radioactivity remaining as residue in the carcass accounted for 0.7%-0.8% for both sexes. It was notable that the concentration of radioactivity in blood and plasma was below the limit of detection at all time points up to 24 hours after dosing. The mean limits of detection were <0.035 μg/g and <0.016 μg/g in blood and plasma, respectively.

It was noted that the rapid excretion of unmetabolised Tinosorb-M in faeces indicates that this radioactivity was not absorbed from the GIT and was not due to absorption followed by elimination in bile. This is supported by the concentration of radioactivity in the blood and plasma being below the limit of detection at all time points over a 24 hours period after dosing. There was no direct measurement of biliary recycling carried out during the study.

In the dermal absorption assay, exposure to a 10% Tinosorb-M formulation for 6 hours resulted in approximately 97% of applied radiolabel remaining the skin surface (determined from material washed off skin at 6 hours). It was noted that approximately 0.2%-0.7% of the applied dose remained associated with the application site following the skin wash and this would be available for absorption. It was suggested that the amount of the dose absorbed remained similar after 6, 24, 72 and 120 hours over a range of 0.2%-0.5%. The absorbed radioactivity was not associated with any specific tissue or organ, with less than 0.01% of the dose found in any tissue at any time point. Less than 0.01% of the dose was excreted in the urine and less than 0.05% of the dose was excreted in the faeces at any time point. Following exposure to 0.2% Tinosorb-M the amounts of radiolabel washed from the skin (97%), remaining in the skin (0.7%) and absorbed through the skin (0.2%-0.8%) were similar to that seen with the 10% dose of Tinosorb-M. In animals treated dermally with Tinosorb-M, since the residues in urine and faeces were very low, no metabolite profiling was conducted.

In general, there were no quantitative differences seen in the kinetic behaviour of Tinosorb-M between the sexes for either the oral or dermal routes. A comparison of the data would indicate that absorption via the dermal route (0.2%-0.8%) would be greater than absorption via the oral route (below detection limit of 0.035 μg/g; less than 0.01% detected in urine). A statement in the study report said that both oral and dermal absorption of labelled Tinosorb M was very low. Analysis of the tissues collected during the study showed only one substance present, which was the parent compound Tinosorb M suggesting no metabolism occurred.

Repeat Dose Toxicity

Dermal Toxicity

90 days dermal toxicity (semi-occlusive) study in the Wistar rat; RCC Ltd Toxicology Division, study no. 795025, Switzerland, for Ciba Specialty Chemicals Inc; May 2002; W. H. Braun; GLP/QA-yes.

In this study, Tinosorb M was administered dermally to groups of SPF Wistar rats (10/sex/dose) at dose levels of 150, 450 or 1000 mg/kg/day applied to shaven skin for a period of 91/92 days. The site of application was re-shaven once a week. Test and control formulations remained on the skin for 6 hours/day, covered by a semi-occlusive dressing. The site of application was kept under a semi-occlusive dressing throughout the study. Two control groups were included in the study; one was an untreated control group and the second control group received a daily dermal application of the vehicle, corn oil. Test and control formulations were prepared for use on a weekly basis. The control groups and the highest dose (1000 mg/kg/day) group included 10 additional animals for assessment after a 4 weeks drug-free recovery period. Parameters monitored included clinical signs of toxicity, treatment site reaction (eg irritation), food consumption, body weight development, ophthalmoscopic examination, haematology, clinical chemistry, necropsy, and macro- and micro-pathology on all control and high dose animals.

Furthermore, plasma levels of Tinosorb M were determined in an additional 9 rats/sex/group over the same dose range of 150, 450 or 1000 mg/kg/day on days 1, 15, 28, 57 and 64 of the treatment period. Sampling took place before daily treatment and at 0.5, 1, 2, 6 and 24 hours after treatment on the designated days.

During the course of the study a total of 3 animals were killed due to their failing physical condition; these animals came from the control (male on day 21), low (female on day 70) dose and high (male on day16) dose groups. These deaths were considered to be unrelated to treatment. Male 29 was sacrificed due to an eye injury and female 199 developed an ear infection. Male 116 displayed reduced activity, spasms and ventral recumbency; microscopic examination of tissues revealed changes (limited to this animal) that were unrelated to treatment with Tinosorb M.

Clinical signs of toxicity were limited to local signs of irritation, which included alopecia and minor skin injuries. These findings were said to be typical of background findings seen in dermal studies performed using semi-occlusive dressings. Similar signs of local irritation were observed in both the control and treatment groups, indicating these adverse effects were not related to treatment. There were no clinical signs of toxicological importance.

There were no overall differences between the treated animals and controls in relative and absolute food consumption or body weight gain during the course of treatment or during the recovery period. There were transient increases in food consumption in some high dose animals, but these were infrequent (males during weeks 5, 7 and 8; females during weeks 1 and 2). Food consumption was similar for all groups during the recovery period. These transient increases in food consumption had no obvious effect on body weights, with body weight gain shown to be similar across all groups during the course of the treatment and recovery periods.

Ophthalmoscopic examination of all groups was unremarkable, with minor effects considered to be unrelated to dermal treatment with Tinosorb M. During the pretreatment screening period a general range of spontaneous conditions were detected, which were also seen in treated animals and control at the of the study. These conditions included corneal or lenticular opacities, persistent pupillary membrane or persistent hyaloid vessels, which were seen at a similar frequency in treatment groups and controls.

Analysis of haematology data revealed no difference between results for the treated animals when compared with the controls. Transient fluctuations in mean corpuscular haemoglobin concentration were observed in males across the dose groups, but the change was slight, not dose-related, not seen in females and no longer evident during the recovery period. Haemoglobin concentration and mean corpuscular haemoglobin were not significant altered at any time point during the study.

Assessment of clinical chemistry revealed slight transient fluctuations in glucose and LDH in males only, and cholesterol, chloride and protein in females only; these changes were seen in a single sex, were not dose-related and were not present during the recovery period. Urinalysis was unremarkable.

An evaluation of the functional performance of the oestrus cycle in the female rats indicated that treated females were unaffected when compared with the controls. The number of oestrus cycles (2.0-2.7) during a 14 days period was not significantly different (no dose-related trends) between the study groups.

Hormone analysis was targeted, with testosterone, oestradiol, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH) and luteinising hormone (LH) all measured during the course of the study. There were no treatment-related changes in any of these hormones, with measured values similar in treated animals and controls.

Organ weight analysis revealed similar mean absolute and relative organ weights for treated animals and controls. There were no apparent treatment-related effects on organ weights following 3 months dermal exposure to Tinosorb M; a similar finding was recorded at the end of the recovery period. One male treated at the highest dose level had markedly higher kidney weight due to the presence of large grey-white nodules in the right kidney. This value was excluded from the organ weight calculation.

Macroscopic and microscopic examination of tissues was unremarkable. A number of macroscopic findings were noted, but they occurred at a similar frequency in treated animals and controls. The adverse findings were associated with the application site and included hyperkeratosis and acanthosis, which were suggested to be due to mechanical irritation of the fur clipping and daily semi-occlusive dressing. Microscopic findings were evenly distributed between the treated animals and controls, and were not associated with treatment with Tinosorb M.

Plasma levels of Tinosorb M were below the level of detection at all time points after the initial treatment period of 6 hours, with no evidence that the test agent was absorbed through the skin at any time point. On the second day of exposure an untreated control male, a vehicle control female, 2 females at the low dose, 4 females at the mid dose, and 1 male and 1 female at the high dose all showed trace amounts of the test material. These findings were considered to be due to inadvertent contamination of collected samples. Contamination was again

evident on sampling days 15 and 28, with one vehicle control recording the greatest plasma value (107.8 ng/mL) seen during the plasma level evaluation. Further evidence of contamination was evident on the last days (57-64) of sampling, with untreated and vehicle controls displaying measurable amounts of the test material. The validity of this process/results was questionable since there was an obvious problem with contamination of samples with the test substance, which complicated the analysis.

Overall, dermal administration of Tinosorb M to Wistar rats at doses of 150, 450 or 1000 mg/kg/day for a period of 3 months was without significant toxicity, with no deaths or clinical signs of toxicity. Local irritation was attributed to mechanical irritation caused by the semi-occlusive dressing used to protect the test agent from being removed by the animal; this reasoning was based on the finding of similar degrees of local irritation in the control and treatment groups. There were no treatment-related adverse effects observed for any of the parameters examined during the course of the study. A NEL of 1000 mg/kg/day was established for the dermal application of Tinosorb M in this study. It is worth noting that the dermal route is the most appropriate route for testing since it the proposed route for use in humans.

PREVIOUSLY EVALUATED DATA (also see attachment 5)

Local Tolerance

Investigation of photostability of UV-absorbers for cosmetic sunscreens (Herzog, B & Sommer, K; Ciba Specialty Chemical Inc., Consumer Care, Germany).

This study examined the photo-stabilities of different UV-filters (formulations containing one UV-filter or mixtures of two different UV-filters), which are investigated using irradiation and subsequent analysis in terms of recovery of the absorber molecules using HPLC and in terms of sunscreen performance using in vitro SPF measurements, respectively. Agents used in this study were two UV-A filters, butyl methoxydibenzoylmethane (BMDBM) and 2,2'-methylene-bis-6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol (MBBT), and two UV-B filters, octyl methoxycinnamate (OMC) and 4-methylbenzylidene camphor (MBC), as well as a UV-boardband absorber bis-octoxyphenol methoxyphenyl triazine (BOMT). These substances were formulated in either oil and water phases. HPLC-analysis and in vitro SPF analysis were used in this assay. Filter concentrations were chosen in a range consistent with levels used in commercial sunscreen formulations; BMDBM 2.4%, OMC 3.4%, MBC 2.4%, BOMT 2.0%, MBBT 5.0%, all w/w). Irradiation was based on values corresponding to UV intensities related to MED/hour doses. The range of dosing was 0, 5, 10, 20 and 50 MED.

The results showed that recovery (expressed as % of initial amount) of the individual filters varied dramatically at 50 MED, with the order from greatest recovery to lowest as follows; MBBT>BOMT>MBC>OMC>BMDBM. Approximately 98% of MBBT was recovered, while approximately 5% of BMDBM was recovered. Recovery of MBBT varied from 95% to 99% over the range of MED intensities. The recovery of BOMT was only 5 to 10% below that of MBBT. When BMDBM was tested in combination with the other UV-filters its recovery improved with all except OMC. Recovery of OMC was enhanced when in combination with MBBT and BOMT.

Measurement of *in vitro* SPF values for BMDBM in combination with the other UV-filters showed a dramatic loss of protection with the combination of BMDBM and OMC, while combinations of BMDBM + MBBT and BMDBM + BOMT maintained protection (and UVA/UVB-ratio) over the range of 5-50 MED. Measurement of *in vitro* SPF values for OMC in combination with other UV-filters showed a reduction in protection up to 10 MED, with no further decline up to 50 MED for OMC + MBBT and OMC + BOMT. Combinations of OMC + MBBT and OMC + BOMT showed an improved UVA/UVB-ratio as the MED increased to 50.

Skin permeation of testosterone *in vitro* and *in vivo*. (An-eX analytical services Ltd, report no. AN/1/99, 1999).

This study examined the *in vitro* skin permeation of testosterone and compared the result with published data on the *in vivo* skin permeation. The formulation, application procedure and dose of chemical applied to the skin *in vitro* were designed to mimic the parameters used in the published *in vivo* protocol. Testosterone was applied to skin taken from four donors and spread evenly at a concentration of 1 $\mu g/cm^2$. A total of 12 replicates were carried out. After 24 hr the formulation was removed from the skin surface using a wiping and washing

procedure. Permeation of testosterone through the skin was monitored by removing samples from the receptor phase at 1, 4, 24, 48, 96 and 120 hr after drug application.

The *in vitro* absorption of testosterone rose steadily over the first 24 hr until it reached a plateau at a value of 33±7% of the applied dose. The literature *in vivo* permeation of testosterone from an analogous formulation under the same exposure conditions amounted to 49.2±4.7% of the applied dose. It was stated that the *in vitro*/*in vivo* ratio was 0.7, and the *in vitro* (AneX)/ *in vitro* (literature) ratio was ratio. The conclusion was that the *in vitro* premeation method acted as a good predictor for the *in vivo* situation.

Comment: A value of 0.7 (0.68 actually) does not appear to be a good predictor of an event.

Reproductive toxicity

Developmental/prenatal toxicity with Tinosorb M (RCC, Research and Consulting Company Ltd., Switzerland, Project no. 680771, Dr U. Mentzel, Aug. 1998, GLP/QA-yes).

Groups (22/dose) of mated female WIST HanIbm (SPF quality) rats were treated with doses of 0, 100, 300 or 1000 mg/kg/day Tinosorb M (by gavage) from day 6 through to day 17 post-coitum. Acclimatised females had been placed in cages with sexually mature males until evidence of copulation (mating day designated day 0 post-coitum) was observed. Dams were sacrificed on day 21 post-coitum and the foetuses removed for examination by caesarean section. The vehicle control (dose group 0) received bi-distilled water containing 0.5% carboxymethylcellulose and 0.1% Tween 80. A standard dose volume of 10 mL/kg was used, with daily adjustments to correct for changing body weight. Doses used in this study were based on dose range-finding study.

There was no evidence of maternal toxicity (clinical signs, food consumption, body weight change, necropsy findings all unaffected) and all animals survived to scheduled sacrifice. In was noted that three mated females were not pregnant (1 from LD and 2 from MD groups), resulting in a reduction of group numbers. Furthermore, an additional female in the LD group had only resorptions further reducing the final number of dams for analysis to 22, 20, 20 and 22 for the control, LD, MD and HD groups, respectively. Isolated occurrence (only in LD group) of females with total resorption is a common finding and was therefore considered not to be treatment-related. Reproductive parameters examined (mean number of implantation sites, mean post-implantation loss, mean number of foetuses per dam) were similar in the treatment groups and controls.

Data generated for the foetuses showed that there were no adverse effects associated with treatment. The mean foetal body weights (almost identical between groups) and the sex ratios for all study groups were similar. There were no observable external, visceral or skeletal abnormalities that could be attributed to treatment with Tinosorb M. Omphalocele, which is a congenital herniation of viscera into the base of the umbilical cord, was noted in 1/235 foetuses from group 2. There were no abnormal findings in 268, 241 and 261 foetuses from groups 1, 3 and 4, respectively. A small number of abnormal findings (predominantly mis-shaped sternebrae [9], also wavy ribs [1] and fused ribs [1]) were observed at a similar frequency in each group. The frequency of abnormal findings was 2/140, 1/122, 4/125 and 4/135 for the control, LD, MD and HD groups, respectively. Skeletal development (variation in ossification) was similar for all the groups in the study.

Based on the results an NEL for both maternal and foetal toxicity was determined to be 1000 mg/kg.

Genotoxicity studies

Data submitted addressing potential genotoxic activity included 1 new study and 3 previously evaluated studies (see appendix). Results from the previously evaluated studies showed that Tinosorb M was not mutagenic (Ames test) in bacterial cells and was not photomutagenic also in bacterial cells. Results from a chromosomal aberration assay using Chinese hamster ovary cells were negative.

Chromosome aberration assay *in vitro*: Photomutagenicity in Chinese hamster V79 cells (Lab:- RCC Cytotest Cell Research, Dr A. Czich, Project no. 615905, Oct. 1998, GLP/QA-yes).

Tinosorb M (1% in DMSO) was assessed for a potential to induce structural chromosome aberrations in Chinese hamster V79 cells in the presence and absence of UVA and UVB irradiation (from Xenon light source). Initially, the test substance was incubated with cultures of CHO cells during irradiation with 200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB. In a second experiment, the dose of radiation was increased by 50% for both UVA and UVB exposure. Preparation of the cells for analysis took place 18 and 24 hr after initiation of radiation. A total of 100 metaphases/culture were scored for structural chromosomal aberrations. The dose used in these assays was based on the previous study using CHO cells and OECD guidelines (on the solubility of the test substance). Negative (and solvent DMSO control) and positive (EMS and 8-methoxypsoralen) control groups were included in the assays.

In both assays, there was no effect on the mitotic indices or on the cell numbers in the presence and absence of UV radiation. It was also shown that the test article did not increase the frequency of cells carrying structural chromosomal aberrations in the presence and absence of UV radiation. Values obtained for the incidence of chromosomal aberrations were similar to those seen with the concurrent negative control, and in the range for the historical control data for the testing laboratory. In both assays, there was no significant increase in the frequency of polyploid metaphases in treatment groups compared to controls. These results indicate that Tinosorb M does not induce chromosomal aberrations in CHO cells, and exposure to UVA and UVB radiation does not result in an increased incidence of chromosomal aberrations (not photomutagenic).

Clinical Data

Study 1. Evaluation of phototoxicity in humans (Lab. Hill Top Research, New Jersey, US, Project no. 100239A, June 1998, A. Parisse, GCP/QA-yes)

It was stated that the method used in this study was a modification of the repeated insult patch test. This study evaluated the test material (10% in formulation) for the induction of phototoxicity by a single application to the skin of human volunteers (28 completed the study). Each subject received duplicate patches; 2 patches with 0.2 mL of the test agent in formulation and two additional control patches (placebo formulation on and saline on the other) positioned on both sides of the spine on naive sites. Patches were left in place for a 24 hr contact period and on removal the sites were mapped (clearly marked) and irradiated with 16 J/cm^2 UVA and 0.75 MED UVB. The MED (minimum erythema dose) had been

determined for each subject prior to commencement of the study. Irradiation was confined to single test and control sites on each subject leaving the remaining sites as non-irradiated controls. All sites were evaluated for responses at approximately 1, 24, 48 and 72 hr following irradiation and patch removal.

Tabulated data were presented, with possible response outcomes identified by code. The results were based on examination looking for grades of erythema (slight, mild, moderate, severe), papule, vesicle, fluid filled lesions, spreading of reaction, weeping, induration, glazing, peeling, scab formation, hyper-pigmentation, hypo-pigmentation and fissuring.

"It was stated in the report that there were no adverse events reported during the conduct of the study. It was also stated that the investigators found very few dermal responses with a score of grade 1 (mild erythema, pink tinge) or higher, and that the test articles were relatively non-irritating. The formulation containing Tinosorb M and the placebo did not show evidence of eliciting a phototoxic response in 28 human subjects." Statistical analysis (none presented) was not carried out on the data. There was no detailed assessment of data, with discussion of the results limited to the summarised statements above.

The following analysis by the evaluator is based on tabulated data presented in the report. Results for subjects from the saline control group showed a slight increase in responses following irradiation compared to no irradiation. Overall, 54% of non-irradiated subjects showed some reaction at the application site at 1 hr, but no further reactions were observed after 1 hr. A total of 68% of irradiated subjects showed a reaction at 1 hr, with a total of 6 and 3 subjects in the irradiated group displayed slight, confluent or patchy erythema at 24 and 48 hr after treatment, respectively. Subjects from the non-irradiated group did not display erythema after the examination at 1 hr post-treatment, except for 1 subject showing slight erythema at 48 hr only. A skin response identified as mild erythema occurred on 5 non-irradiated subjects and 10 irradiated subjects at 1 hr post-treatment only. One subject from the non-irradiated group displayed moderate erythema at 1 hr. There was no incidence of moderate erythema seen on any irradiated subjects.

Results for subjects from the placebo (formulation minus active) control group showed a slight increase in responses following irradiation compared to no irradiation. Overall, 54% of non-irradiated subjects showed some reaction at the application site at 1 hr, while slight erythema was observed at 24, 48 and 72 hr in 3, 1 and 1 subjects, respectively. In the non-irradiated group, mild erythema was observed on 3 subjects at 1 hr only, and moderate erythema was seen on 1 subject at 1 hr only. A total of 71% of irradiated subjects showed a reaction at 1 hr, while slight erythema was observed at 24, 48 and 72 hr in 13, 5 and 3 subjects, respectively. In the irradiated group, mild erythema was observed on 8, 3 and 1 subjects at 1, 24 and 48 hr, respectively. There was no incidence of moderate erythema seen on any irradiated subjects.

Results from subjects from the test (formulation with active) group showed a slight increase in responses following irradiation compared to no irradiation. Overall, 29% of non-irradiated subjects showed some reaction at the application site at 1 hr, while slight erythema was observed at 24, 48 and 72 hr in 3, 2 and 1 subjects, respectively. Mild erythema was observed in 3 subjects at 1 hr, but not at any following examination. A total of 50% of irradiated subjects showed a reaction at 1 hr, while slight erythema was observed at 24, 48 and 72 hr in 11, 2 and 1 subjects, respectively. Mild erythema was observed in 3 subjects at 1 hr, 1 at 24 hr and 1 at 48hr.

Overall, the only consistent adverse findings other than erythema were papule (red, solid pinpoint elevation) and spreading (reaction beyond pad area), which were evident in all groups at irradiated and non-irradiated sites at a similar frequency (in 1-4 subjects from 1-24 hr). There were no other adverse effects noted in the result tables. There was no evidence of systemic toxicity reported by the investigators. Variation in the incidence of erythema did not appear to be associated with irradiation and treatment with the active. The incidence of erythema was increased in the irradiated subjects compared with the non-irradiated subjects. The irradiated test group subjects had a lower incidence of erythema compared with subjects from the irradiated placebo and saline controls groups. A summary of the overall incidence of erythema is presented in the following table.

Frequency of erythema (at 1 hr post treatment)

Subjects UV exposure	Saline control group	Placebo formulation group	Active formulation group (test)
Non-irradiated	54%	54%	29%
Irradiated	68%	68%	50%

Note: These figures include erythema of all intensities observed (slight – moderate)

The incidence of mild erythema was greater in all irradiated groups and it persisted for longer in irradiated groups compared with non-irradiated groups. There was no incidence of moderate erythema in subjects in the test group that were irradiated. The test material (Tinosorb M) appeared to be less active than the placebo and saline control groups. Increased irritation was consistently seen in all irradiated groups, implying the extent of irradiation caused minimal skin damage.

Comments: A lack of statistical analysis and discussion/interpretation of results indicates a degree of qualitative rather than quantitative assessment for this study. Also, the formulation used as a placebo varied slightly from the formulation containing the active. The additional excipients in the active formulation may have provided a soothing function to reduce the intensity of skin irritation caused by the active? The placebo formulation was as follows:

2.5 % propylene glycol stearate SE
5.0% mineral oil
1.5% stearate acid
0.4% cetrearyl alcohol
0.1% propylparaben
85.6% water
0.4% triethanolamine
4.0% glycerine 85%
0.1%methylparaben
0.2% carbomer
0.2% citric acid 50%

The formulation containing the active had these compounds shown above in the same amounts, as well as:

10% Tinosorb M
1.5% decyl glucoside
0.04% xanthan gum

0.08% propylene glycol

Study 2. Evaluation of photoallergy in humans (Lab. Hill Top Research, New Jersey, US, Project no. 100239B, June 1998, A. Parisse, GCP/QA-yes)

It was stated that the method used in this study was an adaptation of the repeat insult patch test. This study evaluated the test material (10% in formulation) for the induction of photoallergy following repeated application to the skin of human volunteers (26 completed the study, 23 F/3M). Each subject received duplicate patches; 2 patches with 0.2 mL of the test agent in formulation and two additional control patches (placebo formulation on and saline on the other) positioned on both sides (for irradiated and non-irradiated assessment) of the spine on naive sites. The induction phase involved 6 applications of patches (each in place for 24 hr) over 3 weeks (2/week). After removal (24 hr after application) of the patches three application sites (1 from each grouping) were irradiated within 10 minutes with 2 x MED UVB/UVA. Three sites (1 from each grouping) were left as non-irradiated controls. The MED had been established for the subjects prior to the study.

Following the induction period, the subjects were not exposed to the test substance or UV radiation for a 2 weeks period. After 2 weeks a challenge application of patches containing either 0.2 mL test article, 0.2 mL placebo (formulation without active) or 0.2 mL of saline were applied to separate naive sites on either side of a subjects spine. After a period of 24 hr the patches were removed and the treated sites irradiated with 16 J/cm^2 of UVA and 0.75 MED of UVB. Evaluation involved inspection of all sites (non-irradiated and irradiated sites) at 1, 24, 48 and 72 hr following UVA and UVB irradiation or after patch removal from non-irradiated sites. A rechallenge (same protocol as initial challenge) was carried out on selected subjects if required to evaluate possible false positive or negative reactions. Statistical analysis of the data was not carried out, with results presented in tabular format.

Adverse events occurring during the study included reported severe burning and itching by a subject that was associated with the test article, which was resolved by applying Aclovate cream without sequelae. A second subject complained of lower back pain, but this complaint was not related to treatment.

The data showed that almost all subjects receiving the placebo displayed erythema (slight to moderate) at some stage of the induction phase. This result is not surprising since irradiation during induction was stated to be at 2 x MED. The following table summarises the results from the placebo group (irradiated). The figures in the table are the number (range) of subjects that presented with the response type (erythema) over the induction period (evaluated at 6 time points) and challenge period (evaluated at 4 time points).

Number of subjects with response type over periods of induction and challenge

Response type	Induction (days 1-6)	Challenge (1-72 hours)
No visible reaction	1-2	2-18
Slight erythema	7-14	6-15
Mild erythema	11-14	1-8
Moderate erythema	3-6	0-1
Severe erythema	0-2	0

The challenge period produced less erythema (both intensity and frequency) than the induction period. Other adverse events included hyper-pigmentation, peeling, spreading and

papule, which occurred in a greater number of subjects during the induction (20/26) phase when compared to the challenge (7/26) phase.

The data showed that almost all subjects receiving the saline control displayed erythema (slight to moderate) at some stage of the induction phase. This result is not surprising since irradiation during induction was stated to be at 2 x MED. The following table summarises the results from the placebo group (irradiated).

Number of subjects with response type over periods of induction and challenge

Response type	Induction (days 1-6)	Challenge (1-72 hours)
No visible reaction	2-7	4-19
Slight erythema	7-16	5-14
Mild erythema	4-11	1-7
Moderate erythema	2-5	0-1
Severe erythema	0-1	0

The challenge period produced less erythema (both intensity and frequency) than the induction period. Other adverse events included hyper-pigmentation, peeling, spreading and oedema, which occurred in a greater number of subjects during the induction (19/26) phase when compared to the challenge (4/26) phase.

The data showed that almost all subjects receiving the test material (Tinosorb M) displayed erythema (slight to moderate) at some stage of the induction phase. This result is not surprising since irradiation during induction was stated to be at 2 x MED. The following table summarises the results from the placebo group (irradiated).

Number of subjects with response type over periods of induction and challenge

Response type	Induction (days 1-6)	Challenge (1-72 hours)
No visible reaction	1-8	8-21
Slight erythema	10-17	4-12
Mild erythema	5-10	1-5
Moderate erythema	0-4	0-1
Severe erythema	0-1	0

The challenge period produced less erythema (both intensity and frequency) than the induction period. Other adverse events included hyper-pigmentation, peeling, spreading and papule, which occurred in a greater number of subjects during the induction (13/26) phase when compared to the challenge (3/26) phase.

Examination of the non-irradiated sites (over the challenge period of 1-72 hr) showed that the skin was almost totally devoid of adverse effects with no erythema observed after the 1 hr inspection. At the 1 hr assessment, slight erythema was observed in 5/26 subjects from the placebo control group, 6/26 from the saline control group and 6/26 from the test group. In the saline control group, 1 subject presented with mild erythema at 1 hr, which was not observed at any later examination.

Despite a lack of statistical analysis, the conclusion that Tinosorb M did not behave as a photo-allergen appears justified when comparing the results for the challenge period for the test material, placebo and saline control groups. The test material generated a similar

response (erythema and other skin reactions) profile to the saline and placebo controls over the course of the study.

Report submitted to MEC in June 1999

This is an application for approval of Tinosorb M a new sunscreen substance. If the substance is approved it will be included in the list of active sunscreen agents which can be used in listed products. Listed sunscreen products need to comply with the Australian and New Zealand Standard (AS/NZS 2604:1998 Sunscreen Products – Evaluation and Classification), which includes a test to establish the efficacy of the product (SPF – sun protection factor) as a sunscreen. The following overview addresses the accompanying evaluation report (compiled by the Chemicals Assessment Section). Tinosorb M has been used in paint products for its primary activity as an UV-A absorber.

This product has not been approved for use in/on humans for any purpose to date. The sponsor indicated that an application has been made to the EC for approval of Tinosorb M as a sunscreen, but requested information on this application has not been presented to the TGA.

The absence of pharmacokinetic/toxicokinetic (in particular in vivo dermal and oral absorption data) data limits the usefulness of the submitted toxicity studies. How much of the test material is absorbed following application to the skin? Do the results from the toxicity studies reflect a lack of toxicity or a lack of absorption from the GIT?

Tinosorb M (500 mg dose) was shown to be a slight skin irritant (increased erythema and oedema) to the skin of rabbits. What is the likelihood that this product will cause skin irritation in humans, given the skin is the primary site of application. Irritated and inflamed skin would have an increased blood flow, which could result in a greater level of absorption of the active substance.

An absence of reproductive toxicity data, in particular developmental data, was seen as a deficiency. Does the committee feel this deficiency needs to be addressed or is this dependent on in vivo absorption data defining the likely systemic exposure?

The evaluator raised the point that the genotoxic/carcinogenic potential had not been fully explored. It was suggested that additional in vivo genotoxicity studies should be carried out and submitted for evaluation. Does the committee agree with the appraisal of the evaluator; could this issue be influenced by the level of systemic exposure?

Skin damage caused by the sun results from exposure to UV-B primarily, and UV-A to a lesser extent. Interestingly, UV-C has the potential to cause more damage than UV-B, but its inability (possibly subject to change) to penetrate the ozone layer has negated the need for UV-C protection. Tinosorb M is an UV-A absorber and a product containing this active would lack UV-B protection, unless an UV-B absorber was included. Does the committee think drug interaction data with any UV-B absorber is required, and is efficacy data (SPF) required for the individual component absorbers, as well possible combinations?

A copy of the Australian/New Zealand Standard for Sunscreen (abbreviated – minus appendix) and a list of sunscreening agents currently permitted as active ingredients are included with the Chemicals Assessment Section report on Tinosorb M.

The Committee is requested to advise on the acceptability of this substance for use as an ingredient in listed sunscreen products.